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(54) Title: SUNFLOWER ANTI-PATHOGENIC PROTEINS AND GENES AND THEIR USES

(57) Abstract: Compositions and methods to aid in protecting plants from invading pathogenic organisms are provided. The compositions of the invention comprise anti-pathogenic genes, including 5' regulatory sequences in the promoter, and proteins encoded by the anti-pathogenic genes. The compositions find use in methods for reducing or eliminating damage to plants caused by plant pathogens. Transformed plants, plant cells, tissues, and seed having enhanced disease resistance are also provided.

SUNFLOWER ANTI-PATHOGENIC PROTEINS AND GENES AND THEIR USES

FIELD OF THE INVENTION

The invention relates to nucleotide sequences and proteins for anti-pathogenic agents and their uses, particularly the genetic manipulation of plant with genes that enhance disease resistance. Promoter sequences are also provided.

BACKGROUND OF THE INVENTION

Plant diseases are often a serious limitation on agricultural productivity and have therefore influenced the history and development of agricultural practices. Only recently have Mendelian genes controlling disease resistance been isolated, and elucidation of their biochemical functions remains a major challenge.

Plant disease outbreaks have resulted in catastrophic crop failures that have triggered famines and caused major social change. Generally, the best strategy for plant disease control is to use resistant cultivars selected or developed by plant breeders for this purpose. However, the potential for serious crop disease epidemics persists today, as evidenced by outbreaks of the Victoria blight of oats and southern corn leaf blight.

Mendelian genetics of resistance to disease in plants is well known. Resistance is often controlled by a single gene, either dominant, semidominant, or recessive. In some instances, multigenes are involved. However, the biochemical mechanisms for gene products involved in plant resistance are known in only a few model cases.

Among the causal agents of infectious diseases of crop plants, phytopathogenic fungi play the dominant role not only by causing devastating epidemics, but also through the less spectacular although persistent and significant annual crop yield losses that have made fungal pathogens a serious economic factor. All of the species of flowering plants are attacked by pathogenic fungi. Generally, however, a single plant species can be host to only a few fungal species, and similarly, most fungi have a limited host range.

To colonize plants, fungal microorganisms have evolved strategies to invade plant tissue, to optimize growth in the plant, and to propagate. Bacteria and viruses, as well as some opportunistic fungal parasites, often depend on natural openings or wounds for invasion. In contrast, many true phytopathogenic fungi have evolved mechanisms to actively traverse the plant's outer structural barriers, the cuticle and the epidermal cell wall. To gain entrance, fungi generally secrete a cocktail of hydrolytic enzymes.

Despite the large number of microorganisms capable of causing disease, most plants are resistant to any given pathogen. The defense mechanisms utilized by plants can take many different forms, ranging from passive mechanical or preformed chemical barriers, which provide non-specific protection against a wide range of organisms, to move more active host-specific responses that provide host- or varietal-specific resistance. Resistance (R) genes are effective against individual pathogen varieties. These genes have been employed in breeding programs upon discovery.

A hypersensitive response (HR) that is elaborated in response to invasion by all classes of pathogens is the most common feature associated with active host resistance. In most cases, activation of the HR leads to the death of cells at the infection site, which results in the restriction of the pathogen to small areas immediately surrounding the initially infected cells. At the whole plant level, the HR is manifested as small necrotic lesions. The number of cells affected by the HR is only a small fraction of the total in the plant, so this response obviously contributes to the survival of plants undergoing pathogen attack.

In plants, robust defense responses to invading phytopathogens often conform to a gene-for-gene relationship. Resistance to a pathogen is only observed when the pathogen carries a specific avirulence (avr) gene and the plant carries a corresponding resistance (R) gene. Because avr-R gene-for-gene relationships are observed in many plant-pathogens systems and are accompanied by a characteristic set of defense responses, a common molecular mechanism *avr-R* gene mediated resistance has been postulated. Thus, disease resistance results from the expression of a resistance gene in the plant and a corresponding avirulence gene in the pathogen and is often associated with the rapid, localized cell death of the hypersensitive response. R genes that respond to specific bacteria, fungal, or viral

pathogens have been isolated from a variety of plant species and several appear to encode cytoplasmic proteins. It has been unclear how such proteins could recognize an extracellular pathogen. Many strategies for plant disease control have been attempted. Resistant cultivars has been selected or developed by plant breeders for disease control. Resistance is especially important for major crops such as the cereals, sugar cane, potato, and soybean. The limitation in use of disease resistance in modern agriculture is adaptability by pathogens to overcome resistance.

The development of new strategies to control diseases is the primary purpose of research on plant/pathogen interactions. These include, for example, the identification of essential pathogen virulence factors and the development of means to block them, or the transfer of resistance genes into crop plants from unrelated species. An additional benefit is a better understanding of the physiology of the healthy plant through a study of the metabolic disturbances caused by plant pathogens.

SUMMARY OF THE INVENTION

Anti-pathogenic compositions and methods for their use are provided. The compositions comprise anti-pathogenic proteins and their corresponding genes and regulatory regions. Particularly, sunflower PR5-1, defensin, and berberine bridge enzyme (BBE) homologues, and fragments and variants thereof, are provided.

The compositions are useful in protecting a plant from invading pathogenic organisms. One method involves stably transforming a plant with a nucleotide sequence of the invention to engineer broad spectrum disease resistance in the plant. The nucleotide sequences will be expressed from a promoter capable of driving expression of a gene in a plant cell. A second method involves controlling plant pathogens by applying an effective amount of an anti-pathogenic protein or composition of the invention to the environment of the pathogens. Additionally, the nucleotide sequences of the invention are useful as genetic markers in disease resistance breeding programs.

Promoters of the genes of the invention find use as disease or pathogen-inducible promoters. Such promoters may be used to express other coding regions,

particularly other anti-pathogenic genes, including disease and insect resistance genes.

The compositions of the invention additionally find use in agricultural and pharmaceutical compositions as antifungal and antimicrobial agents. For agricultural purposes, the compositions may be used in sprays for control of plant disease. As pharmaceutical compositions, the agents are useful for antibacterial and antimicrobial treatments.

The methods of the invention find use in controlling pests, including fungal pathogens, viruses, nematodes, insects, and the like. Transformed plants, plant cells, plant tissues, and seeds, as well as methods for making such transformed compositions are additionally provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the cDNA cloning strategy. (I) Sunflower cDNA libraries were directionally constructed into pBluescript phagemid using a ZAP-cDNA synthesis kit from Stratagene; (II) oligonucleotide primers (P1 and P3) were used to amplify the 5′ end of a target gene by a rapid amplification of cDNA ends (RACE) method. PCR and the 3′ end of the gene were amplified with P2 and P4 primers; (III) P5 primer was designed at the putative start codon (ATG) or upstream the start codon in order to clone full-length cDNA; (IV) the full-length cDNA of the target gene were amplified by PCR with P5 and P4 primers; and (V) the expected full-length cDNA was inserted into TA vector (Invitrogen) for sequencing. Shaded areas represent cloned regions.

Figure 2 depicts an alignment of the amino acid sequence of PR5-1 (SEQ ID:13) from sunflower with other PR5 or osmatin-like proteins from grape, (Swiss-Prot Accession Nos. P93621, SEQ ID:10; and O04708, SEQ ID:11); soybean, (Swiss-Prot Accession No. P25096, SEQ ID:12); tomato, (Swiss-Prot Accession No. Q01591, SEQ ID:14); and potato, (Swiss-Prot Accession No. P50701, SEQ ID:15). A star indicates that the amino acid at that position is conserved for all aligned sequences, and a dash denotes gaps in alignment.

Figure 3 depicts an alignment of the amino acid sequence of a BBE (SEQ ID:20) from sunflower with other BBE homologues and two possible sunflower carbohydrate oxidases. Sunflower-15 (SEQ ID:17) and -19 (SEQ ID:16)

sequences were reported in WO 98/13478. Other BBE homologues include a reticuline oxidase precursor from California poppy, (Swiss-Prot Accession No. P30986, SEQ ID:19) and a BBE from opium poppy, (Swiss-Prot Accession No. P93479, SEQ ID:18).

Figure 4 depicts an alignment of the amino acid sequence of a sunflower defensin (SEQ ID:24)with other antifungal defensins from garden pea (Swiss-Prot Accession No. Q01784, SEQ ID:25), white mustard (Swiss-Prot Accession No. P30231, SEQ ID:22), radish (Swiss-Prot Accession No. P30230, SEQ ID:21) and *Arabidopsis* (Swiss-Prot Accession No. P30224, SEQ ID:23).

DETAILED DESCRIPTION OF THE INVENTION

Compositions and methods for controlling pathogenic agents are provided. The anti-pathogenic compositions comprise sunflower genes, including their promoters, and proteins. Particularly, the sunflower genes and proteins are selected from PR5-1, defensin, and berberine bridge enzyme (BBE). Accordingly, the methods are also useful in protecting plants against fungal pathogens, viruses, nematodes, insects and the like. Additionally, the compositions can be used in formulation use for their antimicrobial activities.

Additionally, the present invention provides for isolated nucleic acid molecules comprising nucleotide sequences for plant promoters shown in SEQ ID:7, SEQ ID:8, and SEQ ID:9; for nucleotide sequences encoding the amino acid sequences shown in SEQ ID:1, SEQ ID:2, and SEQ ID:3; the nucleic acid molecules deposited in a bacterial host as Patent Deposit Nos. PTA-67, PTA-73, PTA-75, respectively; and the nucleic acid molecule deposited as Patent Deposit No. PTA-560 which comprises the nucleotide sequence shown in SEQ ID:9. Further provided are polypeptides having an amino acid sequence encoded by a nucleic acid molecule described herein, for example those set forth in SEQ ID:4. SEQ ID:5, and SEQ ID:6 those deposited as Patent Deposit Nos. PTA-67, PTA-73, PTA-75, respectively, and fragments and variants thereof.

Plasmids containing the promoter sequences and gene nucleotide sequences of the invention were deposited with the Patent Depository of the American Type Culture Collection, Manassas, Virginia. The following plasmids were deposited: May 13, 1999, pHp 15383 containing BBE cDNA; May 13, 1999, pHp 15384

containing BBE promoter sequence; May 13, 1999, pHp 15385 containing defensin cDNA; August 31, 1999, pHp 16125 containing defensin promoter sequence; May 13, 1999, pHp 15395 containing PR5-1 promoter sequences; and May 14, 1999, pHp 15393 containing PR5-1 cDNA; and assigned Patent Deposit Nos. PTA-73, PTA-74, PTA-75, PTA-560, PTA-76, PTA-67, respectively. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112.

As indicated, the sequences of the invention find use as antifungal agents. Thus, the genes can be used to engineer plants for broad spectrum disease resistance. In this manner, the sequences can be used alone or in combination with each other and/or with other known disease resistance genes.

Additionally, the sequences can be used as markers in studying defense signal pathways and in disease resistance breeding programs. The sequences can also be used as baits to isolate other signaling components in defense/resistance responsiveness and to isolate the corresponding promoter. See, generally, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed.), Cold Spring Harbor Laboratory Press, Plainview, New York.

The invention encompasses isolated or substantially purified nucleic acid or protein compositions. An "isolated" or "purified" nucleic acid molecule or protein, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Preferably, an "isolated" nucleic acid is free of sequences that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%,

10%, 5%, (by dry weight) of contaminating protein. When the protein of the invention or biologically active portion thereof is recombinantly produced, preferably, culture medium represents less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-protein of interest chemicals.

By "anti-pathogenic compositions" is intended that the compositions of the invention are capable of suppressing, controlling, and/or killing the invading pathogenic organism.

By "disease resistance" is intended that the plants avoid the disease symptoms that are the outcome of plant-pathogen interactions. That is, pathogens are prevented from causing plant diseases and the associated disease symptoms. The methods of the invention can be utilized to protect plants from disease, particularly those diseases that are caused by plant pathogens.

The compositions of the invention include isolated nucleic acid molecules comprising the promoter nucleotide sequences set forth in SEQ ID:7, SEQ ID:8 and SEQ ID:9. By "promoter" is intended a regulatory region of DNA usually comprising a TATA box capable of directing RNA polymerase II to initiate RNA synthesis at the appropriate transcription initiation site for a particular coding sequence. A promoter may additionally comprise other recognition sequences generally positioned upstream or 5′ to the TATA box, referred to as upstream promoter elements, which influence the transcription initiation rate. It is recognized that having identified the nucleotide sequences for the promoter regions disclosed herein, it is within the state of the art to isolate and identify further regulatory elements in the 5′ untranslated region upstream from the particular promoter regions identified herein. Thus, for example, the promoter regions disclosed herein may further comprise upstream regulatory elements that confer tissue-preferred expression of any heterologous nucleotide sequence operably linked to one of the disclosed promoter sequences. See particularly Australian Patent No. AU-A-77751/94 and U.S. Patent Nos. 5,466,785 and 5,635,618. Generally with the promoter sequences of the invention, the pattern of expression will be inducible.

The inducible promoter sequences of the present invention, when assembled within a DNA construct such that the promoter is operably linked to a nucleotide sequence of interest, enable expression of the nucleotide sequences in

the cells of a plant stably transformed with this DNA construct. The nucleotide sequence of interest encompasses both homologous and heterologous sequences. By "heterologous nucleotide sequence" is intended a sequence that is not naturally occurring with the promoter sequence. While this nucleotide sequence is heterologous to the promoter sequence, it may be homologous, or native, or heterologous, or foreign, to the plant host. Choice of the promoter sequence will determine when and where within the organism the heterologous nucleotide sequence is expressed. Where gene expression in response to a stimulus is desired, an inducible promoter of the invention is the regulatory element of choice. When using an inducible promoter, expression of the nucleotide sequence is initiated in cells in response to a stimulus. By "stimulus" is intended a chemical, which may be applied externally or may accumulate in response to another external stimulus; a pathogen, which may, for example, induce expression as a result of invading a plant cell; or other factor such as environmental stresses, including but not limited to, drought, temperature, and salinity.

Compositions of the invention also include the nucleotide sequences for three sunflower genes: a sunflower PR5 homologue as set forth in SEQ ID:4; a sunflower defensin homologue as set forth in SEQ ID:6; and, a sunflower BBE homologue as set forth in SEQ ID:5, and the corresponding amino acid sequences for the proteins encoded thereby as set forth in SEQ ID:1, SEQ ID:3 and SEQ ID:2, respectively. These gene sequences may be assembled into a DNA construct such that the gene is operably linked to a promoter that drives expression of a coding sequence in a plant cell. Plants stably transformed with this DNA construct express, either in a constitutive or inducible manner, a protein of the invention. Expression of this protein creates or enhances disease resistance in the transformed plant.

BBE [9S0-reticuline:oxygen oxidoreductase (methylene-bridge-forming), EC 1.5.3.9] is a covalently flavinylated oxidase that is a key enzyme in benzophenanthridine alkaloid biosynthesis in plants (Kutchan *et al.* (1995) *J. Biol. Chem. 270*:24475-24481; Blechert *et al* (1995) *Proc. Natl. Acad. Sci. USA 92*:4099-4105; Dittrich *et al.* (1991) *Proc. Natl. Acad. Sci. USA 88*:9969-9973; Chou *et al.* (1998) *Plant J. 15*:289-300). Members of the alkaloid family are known to have potent pharmacological activities. Berberine, for example, is

currently used as an antibacterial treatment for eye infections in Europe and for intestinal infections in the far East. The benzophenanthridine alkaloid, sanguimarine, is an antimicrobial used in the treatment of peridontal disease in both the United States and Europe (Kutchan *et al.* (1995) *J. Biol. Chem.* *270*:24475-24481). In addition, BBE has anti-*Phytophthora* and anti-*Pythium* activity, as well as carbohydrate oxidase activity (WO 98/13478). The BBE-transgenic plants of the invention have enhanced resistance to pathogens. BBE and several other enzymes in the defense pathway are induced by elicitors. See for example Blechert *et al.* (1995) *Proc. Natl. Acad. Sci. USA 92*:4099-4105; Dittrich *et al.* (1991) *Proc. Natl. Acad. Sci. USA 88*:9969-9973.

A sunflower BBE is disclosed that is regulated by oxalate oxidase (oxox) expression and *Sclerotinia* infection. The cDNA (SEQ ID:5) and promoter (SEQ ID:8) sequences of sunflower BBE are provided. In addition, expression of this BBE in sunflower was up-regulated by oxalic acid, H_2O_2, salicylic acid (SA) and jasmonic acid (JA).

Pathogenesis-related protein-5 (PR5) is one of the 9 classes of PR proteins. PR5 shares sequence similarity with osmotin, thaumatin, and zeamatin proteins (Hu *et al.* (1997) *Plant Mol. Biol. 34*:949-959; Ryals *et al.* (1996) *Plant Cell 8*:1809-1819). PR5 proteins have been characterized from a wide range of plant species in both dicotyledonous and monocotyledonous plants. Although the biological function of PR5 proteins has yet to be established, members of this group have been shown to have antifungal activities against a broad range of fungal pathogens (Hu *et al.* (1997) *Plant Mol. Biol. 34*:949-959; Ryals *et al.* (1996) *Plant Cell 8*:1809-1819); Liu *et al.* (1994) *Proc. Natl. Acad. Sci. USA 91*:1888-1892; Liu *et al.* (1995) *Plant Mol. Biol. 29*:1015-1026; Zhu *et al.* (1995) *Plant Physiol. 108*:929-937). In *Arabidopsis*, the induction of PR5 is SA-dependent. The sunflower PR5-1 gene disclosed herein was regulated by oxox expression and *Sclerotinia*-infection The sunflower PR5-1 promoter contains potential pathogen-responsive *cis*-elements, such as an MRE (MYB recognition element).

Defensins are one class among the numerous types of Cys-rich antimicrobial polypeptides, which differ in length, number of cysteine bonds, or folding pattern (Bomann, H.G. (1995) *Annu. Rev. Immunol. 13*:61-92). Like cecropins, insect defensins are produced in a pathogen-inducible manner by the

insect fat body and secreted in the hemolymph (Huffmann *et al.* (1992) *Immunol. Today 13*:411-415). Mammalian defensins are produced by various specialized cells in the mammalian body (Lehrer *et al.* (1993) *Annu. Rev. Immunol. 11*:105-128; Ganz *et al.* (1994) *Curr. Opin. Immunol. 6*:584-589). The structural and functional properties of plant defensins resemble those of insect and mammalian defensins (Terras *et al.* (1995) *Plant Cell 7*:573-588; Broekaer *et al.* (1995) *Plant Physiol. 108*:1353-1358). Plant defensins inhibit the growth of a broad range of fungi at micromolar concentrations by inhibiting hyphal elongation or inhibiting hyphal extension (Broekaer *et al.* (1995) *Plant Physiol. 108*:1353-1358).

Plant defensins are important components of the defense system in plants. They are located at the periphery of different organs and are induced by pathogens. A sunflower cDNA was isolated that encodes a defensin peptide (SEQ ID:6). This defensin gene was up regulated by *Sclerotinia* infection, oxox expression, oxalic acid, H_2O_2 and SA as well as jasmonic acid. In general, plant defensin genes such as *Arabidopsis* PDF1.2 and a radish defensin are induced by pathogens via an SA-independent and JA-dependent pathway (Thomma *et al.*) *Proc. Natl. Acad. Sci. USA 95*:15107-15111; Terras *et al.* (1995) *Plant Cell 7*:573-588; Terra *et al.* (1988) *Planta 206*:117-124). The sunflower defensin gene appears to be the only defensin that is regulated via a SA-dependent pathway. The sunflower defensin promoter contains potential pathogen responsive cis-elements, such as W-boxes and G-boxes.

The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the proteins can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well-known in the art. See, for example, Kunkel, T. (1985) *Proc. Natl. Acad. Sci. USA 82*:488-492; Kunkel *et al.* (1987) *Methods in Enzymol. 154*:367-382; U.S. Patent No. 4,873,192; Walker *et al.* (1983) (eds.) *Techniques in Molecular Biology*, MacMillan Publishing Company, NY and the references cited therein. Thus, the genes and nucleotide sequences of the invention include both the naturally occurring sequences as well as mutant forms. Likewise, the proteins of the invention encompass both naturally occurring proteins as well as variations and modified forms thereof. Such variants

will continue to possess the desired defense activation activity. Obviously, the mutations that will be made in the DNA encoding the variant must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, for example, EP Patent Application Publication No. 75,444.

Fragments and variants of these native nucleotide and amino acid sequences are also encompassed by the present invention. By "fragment" is intended a portion of the nucleotide or amino acid sequence. Fragments of a promoter nucleotide sequence may retain their regulatory activity. Thus, for example, less than the entire promoter sequences disclosed herein may be utilized to drive expression of an operably linked nucleotide sequence of interest, such as a nucleotide sequence encoding a heterologous protein. It is within skill in the art to determine whether such fragments decrease expression levels or alter the nature of expression, i.e., and constitutive or inducible expression. Alternatively, fragments of a promoter nucleotide sequence that are useful as hybridization probes, such as described below, generally do not retain this regulatory activity.

Nucleic acid molecules that are fragments of a promoter nucleotide sequence comprise at least 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 325, 350, 375, 400, 425, 450, or 500 nucleotides, or up to the number of nucleotides present in the full-length promoter nucleotide sequence set forth in SEQ ID: 7, 8, and 9. Fragments of a promoter sequence that retain their regulatory activity comprise at least 30, 35, 40 contiguous nucleotides, preferably at least 50 contiguous nucleotides, more preferably at least 75 contiguous nucleotides, still more preferably at least 100 contiguous nucleotides of the particular promoter nucleotide sequence disclosed herein. Preferred fragment lengths depend upon the objective and will also vary depending upon the particular promoter sequence.

The nucleotides of such fragments will usually comprise the TATA recognition sequence of the particular promoter sequence. Such fragments may be obtained by use of restriction enzymes to cleave the naturally occurring promoter nucleotide sequence disclosed herein; by synthesizing a nucleotide sequence from the naturally occurring sequence of the promoter DNA sequence; or may be obtained through the use of PCR technology. See particularly, Mullis *et al.* (1987) *Methods Enzymol. 155*:335-350, and Erlich, ed. (1989) *PCR Technology* (Stockton

Press, New York). Variants of these promoter fragments, such as those resulting from site-directed mutagenesis, are also encompassed by the compositions of the present invention.

With respect to the antipathogenic nucleotide sequences, fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native proteins, i.e., the sequences set forth in SEQ IDS 1,2, and 3, and hence enhance disease resistance when expressed in a plant. Alternatively, fragments of a coding nucleotide sequence that is useful as hybridization probes generally do not encode fragment proteins retaining biological activity. Thus, fragments of a nucleotide sequence may range from at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the entire nucleotide sequence encoding the proteins of the invention.

A fragment of an antipathogenic nucleotide sequence that encodes a biologically active portion of a protein of the invention will encode at least 15, 25, 30, 40, 50, 75, 100, or 150 contiguous amino acids, or up to the total number of amino acids present in a full-length protein of the invention. Fragments of a nucleotide sequence of the invention that are useful as hybridization probes for PCR primers generally need not encode a biologically active portion of a protein.

A biologically active portion of a protein of the invention can be prepared by isolating a portion of one of the nucleotide sequences of the invention, expressing the encoded portion of the protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the protein of interest. Nucleic acid molecules that are fragments of a nucleotide sequence of the invention comprise at least 15, 20, 50, 75, 100, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, or 800 nucleotides, or up to the number of nucleotides present in a full-length sunflower homologue nucleotide sequence disclosed herein.

By "variants" is intended substantially similar sequences. For nucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the antipathogenic polypeptides of the invention. Naturally occurring allelic variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also

include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode an antipathogenic protein of the invention. Generally, variants of a particular nucleotide sequence of the invention will have at least 40%, 50%, 60%, 70%, generally at least 75%, 80%, 85%, 87%, preferably about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, and more preferably about 98%, 99% or more sequence identity to that particular nucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters.

By "variant" protein is intended a protein derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, the defense activation activity as described herein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of a native antipathogenic protein of the invention will have at least 40%, 50%, 60%, 70%, generally at least 75%, 80%, 85%, preferably about 90% to 95% or more, and more preferably about 98% or more sequence identity to the amino acid sequence for the native protein as determined by sequence alignment programs described elsewhere herein using default parameters. A biologically active variant of a protein of the invention may differ from that protein by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the antipathogenic proteins can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985) *Proc. Natl. Acad. Sci. USA 82*:488-492; Kunkel *et al.* (1987) *Methods in Enzymol. 154*:367-382; US Patent No. 4,873,192;

Walker and Gaastra (eds.) *Techniques in Molecular Biology*, MacMillan Publishing Company, NY (1983) and the references cited therein.

Thus, the promoters and gene nucleotide sequences of the invention include both the naturally occurring sequences as well as mutant forms. Likewise, the proteins of the invention encompass both naturally occurring proteins as well as variations and modified forms thereof. Such variants will continue to possess the desired promoter activity or antipathogenic defense protein activity. Obviously, the mutations that will be made in the DNA encoding a variant protein must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, for example, EP Patent Application Publication No. 75,444.

In this manner, the present invention encompasses the antipathogenic proteins as well as components and fragments thereof. That is, it is recognized that component polypeptides or fragments of the proteins may be produced which retain antipathogenic protein activity that enhances disease resistance in a plant. These fragments include truncated sequences, as well as N-terminal, C-terminal, internal and internally deleted amino acid sequences of the proteins.

The deletions, insertions, and substitutions of the protein sequences encompassed herein are not expected to produce radical changes in the characteristics of the antipathogenic proteins. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. That is, the activity of the modified protein sequences can be evaluated by monitoring of the plant defense system. See, for example U.S. Patent No. 5,614,395, herein incorporated by reference.

The nucleotide sequences of the invention can be used to isolate corresponding sequences from other organisms, particularly other plants, more particularly other monocots. In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences based on their sequence homology to the sequences set forth herein. Sequences isolated based on their sequence identity to the entire antipathogenic sequences set forth herein or to fragments thereof are encompassed by the present invention.

In a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any plant of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York). See also Innis *et al.*, eds. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, eds. (1995) *PCR Strategies* (Academic Press, New York); and Innis and Gelfand, eds. (1999) *PCR Methods Manual* (Academic Press, New York). Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like.

In hybridization techniques, all or part of a known nucleotide sequence is used as a probe that selectively hybridizes to other corresponding nucleotide sequences present in a population of cloned genomic DNA fragments or cDNA fragments (i.e., genomic or cDNA libraries) from a chosen organism. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P, or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the antipathogenic sequences of the invention. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

For example, the entire antipathogenic sequence disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding antipathogenic sequence or messenger RNAs. Additionally, the promoter sequences described herein, or one or more portions thereof, may be used a as a probe capable of hybridizing to corresponding promoter sequences.

To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among antipathogenic sequences or promoter sequence and are preferably at least about 10 nucleotides in length, and most

preferably at least about 20 nucleotides in length. Such probes may be used to amplify corresponding antipathogenic sequences or promoter sequences from a chosen plant by PCR. This technique may be used to isolate additional coding sequences from a desired plant or as a diagnostic assay to determine the presence of coding sequences in a plant. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Duration of

hybridization is generally less than about 24 hours, usually about 4 to about 12 hours.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem. 138*:267-284: $T_m = 81.5°C + 16.6$ (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq$90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New

York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Thus, isolated sequences that either have promoter activity or encode for a antipathogenic protein and which hybridize under stringent conditions to the sequences disclosed herein, or to fragments thereof, are encompassed by the present invention. Such sequences will be at least 40% to 50% homologous, about 60% to 70% homologous, and even about 75%, 80%, 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% to 98% homologous or more with the disclosed sequences. That is, the sequence identity of sequences may range, sharing at least 40% to 50%, about 60% to 70%, and even about 75%, 80%, 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% to 98% or more sequence identity.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", (d) "percentage of sequence identity", and (e) "substantial identity".

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent sequence identity between any two sequences can be accomplished using a mathematical algorithm. Preferred, non-limiting examples of such mathematical algorithms are the algorithm of Myers and

Miller (1988) *CABIOS 4*:11-17; the local homology algorithm of Smith *et al.* (1981) *Adv. Appl. Math. 2*:482; the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol. 48*:443-453; the search-for-similarity-method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. 85*:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA 872264*, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA 90*:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 8 (available from Genetics Computer Group (GCG), 575 Science Drive, Madison, Wisconsin, USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins *et al.* (1988) *Gene 73*:237-244 (1988); Higgins *et al.* (1989) *CABIOS 5*:151-153; Corpet *et al.* (1988) *Nucleic Acids Res. 16*:10881-90; Huang *et al.* (1992) *CABIOS 8*:155-65; and Pearson *et al.* (1994) *Meth. Mol. Biol. 24*:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) *supra*. A PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul *et al* (1990) *J. Mol. Biol. 215*:403 are based on the algorithm of Karlin and Altschul (1990) *supra*. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a protein or polypeptide of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res. 25*:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs

(e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See http://www.ncbi.nlm.nih.gov. Alignment may also be performed manually by inspection.

For purposes of the present invention, comparison of nucleotide or protein sequences for determination of percent sequence identity to the promoter sequence or the anitpathogenic sequences disclosed herein is preferably made using the Clustal W program (Version 1.7 or later) with its default parameters or any equivalent program. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by the preferred program.

(c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison

window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

(e)(i) The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70% sequence identity, preferably at least 80%, more preferably at least 90%, and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning, and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 95%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. However, stringent conditions encompass temperatures in the range of about 1°C to about 20°C lower than the T_m, depending upon the desired degree of stringency as otherwise qualified herein. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides they encode are substantially identical. This may occur, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is when the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

(e)(ii) The term "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with at least 70% sequence identity to a reference sequence, preferably 80%, more preferably 85%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* *48*:443-453. An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. Peptides that are "substantially similar" share sequences as noted above except that residue positions that are not identical may differ by conservative amino acid changes.

The anti-pathogenic genes and proteins as well as the anti-pathogenic homologue genes and proteins of the invention can also be used to control resistance to pathogens by enhancing the defense mechanisms in a plant. While the exact function of the anti-pathogenic homologues is not known, they are involved in influencing the expression of defense-related proteins. It is recognized that the present invention is not premised upon any particular mechanism of action of the anti-pathogenic genes. It is sufficient for purposes of the invention that the genes and proteins are involved in the plant defense system and can be used to increase resistance levels in the plant to pathogens.

The plant defense mechanisms described herein may be used alone or in combination with other proteins or agents to protect against plant diseases and pathogens. Other plant defense proteins include those described in copending applications entitled "*Methods for Enhancing Disease Resistance in Plants*", U.S. Application Serial No. 60/076,151 filed February 26, 1998, and U.S. Application Serial No. 60/092,464, filed July 11, 1998, and copending application entitled "*Genes for Activation of Plant Pathogen Defense Systems*", U.S. Application Serial No. 60/076,693, filed February 26, 1998, all of which are herein incorporated by reference.

The nucleotide sequences of the invention can be introduced into any plant. The genes to be introduced can be conveniently used in expression cassettes for introduction and expression in any plant of interest.

Such expression cassettes will comprise a transcriptional initiation region linked to the nucleotide sequence of interest. Such an expression cassette is provided with a plurality of restriction sites for insertion of the gene of interest to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The transcriptional initiation region, the promoter, may be native or analogous or foreign or heterologous to the plant host. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. By foreign is intended that the transcriptional initiation region is not found in the native plant into which the transcriptional initiation region is introduced. As used herein a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence.

The transcriptional cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region, a DNA sequence of interest, and a transcriptional and translational termination region functional in plants. The termination region may be native with the transcriptional initiation region, may be native with the DNA sequence of interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens* such as the octopine synthase and nopaline synthase termination regions. See also, Guerineau *et al.* (1991) *Mol. Gen. Genet. 262*:141-144; Proudfoot (1991) *Cell 64*:671-674; Sanfacon *et al.* (1991) *Genes Dev. 5*:141-149; Mogen *et al.* (1990) *Plant Cell 2*:1261-1272; Munroe *et al.* (1990) *Gene 91*:151-158; Ballas *et al.* 1989) *Nuc. Acids Res. 17*:7891-7903; Joshi *et al.* (1987) *Nuc. Acid Res. 15*:9627-9639.

A number of promoters can be used in the practice of the invention. An inducible promoter can be used to drive the expression of the genes of the invention. The inducible promoter will be expressed in the presence of a pathogen to prevent infection and disease symptoms. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc.

See, for example, Rodolfi *et al.* (1983) *Neth. J. Plant Pathol. 89*:245-254; Uknes *et al.* (1992) *Plant Cell 4*:645-656; and Van Loon (1985) *Plant Mol. Virol. 4*:111-116. See, also the copending applications entitled "*Inducible Maize Promoters*", U.S. Application Serial No. 60/076,100, filed February 26, 1998 and U.S. Application Serial No. 60/079,648, filed February 27, 1998, and herein incorporated by reference.

Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau *et al.* (1987) *Plant Mol. Biol. 1*:335-342; Matton *et al.* (1989) *Molecular Plant-Microbe Interactions 2*:325-331; Somsisch *et al.* (1986) *Proc. Natl. Acad. Sci. USA 83*:2427-2430; Somsisch *et al.* (1988) *Molecular and General Genetics 2*:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA 93*:14972-14977. See also, Chen *et al.* (1996) *Plant J. 10*:955-966; Zhang *et al.* (1994) *Proc. Natl. Acad. Sci. USA 91*:2507-2511; Warner *et al.* (1993) *Plant J. 3*:191-201; Siebertz *et al.* (1989) *Plant Cell 1*:961-968; and the references cited therein. Of particular interest is the inducible promoter for the maize PRms gene, whose expression is induced by the pathogen *Fusarium moniliforme* (see, for example, Cordero *et al.* (1992) *Physiol. Mol. Plant Path. 41*:189-200).

Additionally, as pathogens find entry into plants through wounds or insect damage, a wound inducible promoter may be used in the constructions of the invention. Such wound inducible promoters include potato proteinase inhibitor (pin II) gene (Ryan *et al. Ann. Rev. Phytopath. 28*:425-449; Duan *et al. Nature Biotechnology 14*:494-498); wun1 and wun2, U.S. Patent No. 5,428,148; win1 and win2 (Stanford *et al. Mol. Gen Genet 215*:200-208); systemin (McGurl *et al. Science 225*:1570-1573); WIP1 (Rohmeier *et al. Plant Mol. Biol. 22*:783-792; Eckelkamp *et al. FEBS Letters 323*:73-76); MPI gene (Corderok *et al. Plant Journal 6(2)*:141-150); and the like, herein incorporated by reference.

Constitutive promoters include, for example, the Rsyn7 (copending U.S. Application Serial No. 08/661,601), the scp1 promoter (copending U.S. Application Serial No. 09/028,819), the ucp promoter, 35S CaMV promoter, and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142. See also, copending application entitled "*Constitutive Maize*

Promoters", U.S. Application Serial No. 60/076,075, filed February 26, 1998, and herein incorporated by reference.

Tissue-preferred promoters include Yamamoto *et al.* (1997) *Plant J. 12(2)*:255-265; Kawamata *et al.* (1997) *Plant Cell Physiol. 38(7)*:792-803; Hansen *et al.* (1997) *Mol Gen Genet. 254(3)*:337-343; Russell *et al.* (1997) *Transgenic Res. 6(2)*:157-168; Rinehart *et al.* (1996) *Plant Physiol. 112(3)*:1331-1341; Van Camp *et al.* (1996) *Plant Physiol. 112(2)*:525-535; Canevascini *et al.* (1996) *Plant Physiol. 112(2)*:513-524; Yamamoto *et al.* (1994) *Plant Cell Physiol. 35(5)*:773-778; Lam (1994) *Results Probl. Cell Differ. 20*:181-196; Orozco *et al.* (1993) *Plant Mol Biol 23(6)*:1129-1138; Matsuoka *et al.* (1993) *Proc Natl. Acad. Sci. USA 90(20)*:9586-9590; and Guevara-Garcia *et al.* (1993) *Plant J. 4(3)*:495-505.

The nucleotide sequences for the constitutive promoters disclosed in the present invention, as well as variants and fragments thereof, are useful in the genetic manipulation of any plant when assembled within a DNA construct such that the promoter sequence is operably linked with a heterologous nucleotide sequence whose constitutive expression is to be controlled to achieve a desired phenotypic response. By "operably linked" is intended the transcription or translation of the heterologous nucleotide sequence is under the influence of the promoter sequence. In this manner, the nucleotide sequences for the promoters of the invention are provided in expression cassettes along with heterologous nucleotide sequences for expression in the plant of interest. It is recognized that the promoter sequences of the invention may also be used with their native coding sequences to increase or decrease expression of the native coding sequence, thereby resulting in a change in phenotype in the transformed plant.

The promoter nucleotide sequences and methods disclosed herein are useful in regulating expression of any heterologous nucleotide sequence in a host plant in order to vary the phenotype of a plant. Various changes in phenotype are of interest including modifying the fatty acid composition in a plant, altering the amino acid content of a plant, altering a plant's pathogen defense mechanism, and the like. These results can be achieved by providing expression of heterologous products or increased expression of endogenous products in plants. Alternatively, the results can be achieved by providing for a reduction of expression of one or

more endogenous products, particularly enzymes or cofactors in the plant. These changes result in a change in phenotype of the transformed plant.

Genes of interest are reflective of the commercial markets and interests of those involved in the development of the crop. Crops and markets of interest change, and as developing nations open up world markets, new crops and technologies will emerge also. In addition, as our understanding of agronomic traits and characteristics such as yield and heterosis increase, the choice of genes for transformation will change accordingly. General categories of genes of interest include for example, those genes involved in information, such as zinc fingers, those involved in communication, such as kinases, and those involved in housekeeping, such as heat shock proteins. More specific categories of transgenes, for example, include genes encoding important traits for agronomics, insect resistance, disease resistance, herbicide resistance, sterility, grain characteristics, and commercial products. Genes of interest include, generally, those involved in oil, starch, carbohydrate, or nutrient metabolism as well as those affecting kernel size, sucrose loading, and the like.

Agronomically important traits such as oil, starch, and protein content can be genetically altered in addition to using traditional breeding methods. Modifications include increasing content of oleic acid, saturated and unsaturated oils, increasing levels of lysine and sulfur, providing essential amino acids, and also modification of starch. Hordothionin protein modifications are described in U.S. Patent Nos. 5,703,049, 5,885,801, 5,885,802, and 5,990,389; herein incorporated by reference. Another example is lysine and/or sulfur rich seed protein encoded by the soybean 2S albumin described in U.S. Serial No. 08/618,911, filed March 20, 1996, and the chymotrypsin inhibitor from barley, Williamson *et al.* (1987) *Eur. J. Biochem. 165*:99-106, the disclosures of which are herein incorporated by reference.

Derivatives of the coding sequences can be made by site directed mutagenesis to increase the level of preselected amino acids in the encoded polypeptide. For example, the gene encoding the barley high lysine polypeptide (BHL) is derived from barley chymotrypsin inhibitor, U.S. Serial No. 08/740,682, filed November 1, 1996, and PCT/US97/20441, filed October 31, 1997, the disclosures of each are incorporated herein by reference. Other proteins include

methionine-rich plant proteins such as from sunflower seed (Lilley *et al.* (1989) *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, ed. Applewhite (American Oil Chemists Society, Champaign, Illinois), pp. 497-502; herein incorporated by reference)); corn (Pedersen *et al.* (1986) *J. Biol. Chem. 261*:6279; Kirihara *et al.* (1988) *Gene 71*:359; both of which are herein incorporated by reference); and rice (Musumura *et al.* (1989) *Plant Mol. Biol. 12*:123, herein incorporated by reference). Other agronomically important genes encode latex, Floury 2, growth factors, seed storage factors, and transcription factors.

Insect resistance genes may encode resistance to pests that have great yield drag such as rootworm, cutworm, European Corn Borer, and the like. Such genes include, for example Bacillus thuringiensis toxic protein genes (U.S. Patent Nos. 5,366,892; 5,747,450; 5,736,514; 5,723,756; 5,593,881; Geiser *et al.* (1986) *Gene 48*:109); lectins (Van Damme *et al.* (1994) *Plant Mol. Biol. 24*:825); and the like.

Genes encoding disease resistance traits include detoxification genes, such as against fumonisin (U.S. Patent Application Serial No. 08/484,815, filed June 7, 1995); avirulence (avr) and disease resistance (R) genes (Jones et al. (1994) Science 266:789; Martin et al. (1993) Science 262:1432; Mindrinos et al. (1994) Cell 78:1089); and the like.

Herbicide resistance traits may include genes coding for resistance to herbicides that act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance, in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides that act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the *bar* gene), or other such genes known in the art. The *bar* gene encodes resistance to the herbicide basta, the *nptII* gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS gene encodes resistance to the herbicide chlorsulfuron.

Sterility genes can also be encoded in an expression cassette and provide an alternative to physical detasseling. Examples of genes used in such ways include male tissue-preferred genes and genes with male sterility phenotypes such as QM, described in U.S. Patent No. 5,583,210. Other genes include kinases and those encoding compounds toxic to either male or female gametophytic development.

The quality of grain is reflected in traits such as levels and types of oils, saturated and unsaturated, quality and quantity of essential amino acids, and levels of cellulose. In corn, modified hordothionin proteins, described in U.S. Patent Nos. 5,703,049, 5,885,801, 5,885,802, and 5,990,389, provide descriptions of modifications of proteins for desired purposes.

Commercial traits can also be encoded on a gene or genes that could increase for example, starch for ethanol production, or provide expression of proteins. Another important commercial use of transformed plants is the production of polymers and bioplastics such as described in U.S. Patent No. 5,602,321 issued February 11, 1997. Genes such as B-Ketothiolase, PHBase (polyhydroxybutyrate synthase) and acetoacetyl-CoA reductase (see Schubert *et al.* (1988) *J. Bacteriol. 170*:5837-5847) facilitate expression of polyhyroxyalkanoates (PHAs).

Exogenous products include plant enzymes and products as well as those from other sources including procaryotes and other eucaryotes. Such products include enzymes, cofactors, hormones and the like. The level of proteins, particularly modified proteins having improved amino acid distribution to improve the nutrient value of the plant, can be increased. This is achieved by the expression of such proteins having enhanced amino acid content.

Thus, the heterologous nucleotide sequence operably linked to one of the constitutive promoters disclosed herein may be a structural gene encoding a protein of interest. Examples of such heterologous genes include, but are not limited to, genes encoding proteins conferring resistance to abiotic stress, such as drought, temperature, salinity, and toxins such as pesticides and herbicides, or to biotic stress, such as attacks by fungi, viruses, bacteria, insects, and nematodes, and development of diseases associated with these organisms. More particularly, the constitutive promoters disclosed herein and identified as weak constitutive promoters are useful in transforming plants to constitutively express an avirulence gene as disclosed in the copending applications both entitled "*Methods for Enhancing Disease Resistance in Plants*," U.S. Application Serial No. 60/075,151, filed February 26, 1998, and U.S. Application Serial No. 60/092,464, filed July 11, 1998, both of which are herein incorporated by reference. Such weak promoters may cause activation of the plant defense system short of hypersensitive cell death.

Thus, there is an activation of the plant defense system at levels sufficient to protect from pathogen invasion. In this state, there is at least a partial activation of the plant defense system wherein the plant produces increased levels of antipathogenic factors such as PR proteins, *i.e.*, PR-1, cattiness, a-glucanases, etc.; secondary metabolites; phytoalexins; reactive oxygen species; and the like.

Alternatively, the heterologous nucleotide sequence operably linked to one of the constitutive promoters disclosed herein may be an antisense sequence for a targeted gene. By "antisense DNA nucleotide sequence" is intended a sequence that is in inverse orientation to the 5' to 3' normal orientation of that nucleotide sequence. When delivered into a plant cell, expression of the antisense DNA sequence prevents normal expression of the DNA nucleotide sequence for the targeted gene. The antisense nucleotide sequence encodes an RNA transcript that is complementary to and capable of hybridizing to the endogenous messenger RNA (mRNA) produced by transcription of the DNA nucleotide sequence for the targeted gene. In this case, production of the native protein encoded by the targeted gene is inhibited to achieve a desired phenotypic response. Thus the promoter sequences disclosed herein may be operably linked to antisense DNA sequences to reduce or inhibit expression of a native protein in the plant.

The genes and promoters of the invention are provided in expression cassettes for expression in the plant of interest. The cassette will include 5' and 3' regulatory sequences operably linked to the gene of interest. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on another expression cassette. Where appropriate, the gene(s) may be optimized for increased expression in the transformed plant. That is, the genes can be synthesized using plant preferred codons for improved expression. Methods are available in the art for synthesizing plant preferred genes. See, for example, U.S. Patent Nos. 5,330,831, 5,436,391, and Murray *et al.* (1989) *Nuc. Acids Res.* *17*:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences, which may be deleterious to gene

expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein *et al.* (1989) *PNAS USA 86*:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison *et al.* (1986); MDMV leader (Maize Dwarf Mosaic Virus); *Virology 154*:9-20), and human immunoglobulin heavy-chain binding protein (BiP) (Macejak *et al.* (1991) *Nature 353*:90-94; untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4), (Jobling *et al.* (1987) *Nature 325*:622-625; tobacco mosaic virus leader (TMV), (Gallie, D.R. (1989) *Molecular Biology of RNA 237*-256; and maize chlorotic mottle virus leader (MCMV) (Lommel *et al.* (1991) *Virology 81*:382-385). See also, Della-Cioppa *et al.* (1987) *Plant Physiology 84*:965-968. Other methods known to enhance translation can also be utilized, for example, introns, and the like.

In preparing the expression cassette, the various DNA fragments may be manipulated so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Towards this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g. transitions and transversions, may be involved.

The genes of the present invention can be used to transform any plant. In this manner, genetically modified plants, plant cells, plant tissue, seed, and the like can be obtained. Transformation protocols may vary depending on the type of plant or plant cell, *i.e.* monocot or dicot, targeted for transformation. Suitable methods of transforming plant cells include microinjection (Crossway *et al.* (1986) *Biotechniques 4*:320-334), electroporation (Riggs *et al.* (1986) *Proc. Natl. Acad.*

Sci. USA 83:5602-5606, *Agrobacterium* mediated transformation (Hinchee *et al.* (1988) *Biotechnology* 6:915-921), direct gene transfer (Paszkowski *et al.* (1984) *EMBO J.* 3:2717-2722), and ballistic particle acceleration (see, for example, Sanford *et al.*, U.S. Patent 4,945,050; Tomes *et al.* Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment; In Gamborg and Phillips (Eds.) *Plant Cell, Tissue and Organ Culture: Fundamental Methods*, Springer-Verlag, Berlin (1995); and McCabe *et al.* (1988) *Biotechnology* 6:923-926). Also see, Weissinger *et al.* (1988) *Ann. Rev. Genet.* 22:421-477; Sanford *et al.* (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou *et al.* (1988) *Plant Physiol.* 87:671-674(soybean); McCabe *et al.* (1988) *Bio/Technology* 6:923-926 (soybean); Datta *et al.* (1990) *Biotechnology* 8:736-740 (rice); Klein *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 5:4305-4309 (maize); Klein *et al.* (1988) *Biotechnology* 6:559-563 (maize); Tomes *et al.* Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment; In Gamborg and Phillips (eds.) *Plant Cell, Tissue and Organ Culture: Fundamental Methods*, Springer-Verlag, Berlin (1995) (maize); Klein *et al.* (1988) *Plant Physiol.* 91:440-444(maize); Fromm *et al.* (1990) *Biotechnology* 8:833-839 (maize); Hooydaas-Van Slogteren *et al.* (1984) *Nature (London)* 311:763-764; Bytebier *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet *et al.* (1985) In *The Experimental Manipulation of Ovule Tissues* ed. G.P. Chapman *et al.*, pp. 197-209. Longman, NY (pollen); Kaeppler *et al.* (1990) *Plant Cell Reports* 9:415-418; and Kaeppler *et al.* (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D'Halluin *et al.* (1992) *Plant Cell* 4:1495-1505 (electroporation); Li *et al.* (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford (1995) *Annals of Botany* 75:407-413 (rice); Osjoda *et al.* (1996) *Nature Biotechnology* 14:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

The cells, which have been transformed, may be grown into plants in accordance with conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell Reports* 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that the subject phenotypic characteristic is stably

maintained and inherited and then seeds harvested to ensure the desired phenotype or other property has been achieved.

The methods of the invention can be used with other methods available in the art for enhancing disease resistance in plants.

Methods for increasing pathogen resistance in a plant are provided. The methods involve stably transforming a plant with a DNA construct comprising an anti-pathogenic nucleotide sequence of the invention operably linked to promoter that drives expression in a plant. Such methods find use in agriculture particularly in limiting the impact of plant pathogens on crop plants. The anti-pathogenic nucleotide sequences comprise sunflower genes. Particularly, the sunflower genes are selected from the genes encoding PR5, defensin and BBE. While the choice of promoter will depend on the desired timing and location of expression of the anti-pathogenic nucleotide sequences, preferred promoters include constitutive and pathogen-inducible promoters.

Methods are provided for increasing the resistance of a plant to a pathogen involving stably transforming a plant with a DNA construct comprising a nucleotide sequence of an inducible promoter of an antipathogenic gene of the invention operably linked to a second nucleotide sequence. Preferably, the promoter is selected from the promoters of genes encoding a PR5, a BBE homologue or a defensin. More preferably, the promoter has a nucleotide sequence selected from the sequences set forth in SEQ ID:7, SEQ ID:8, and SEQ ID:9. Although any one of a variety of second nucleotide sequences may be utilized, preferred embodiments of the invention encompass those second nucleotide sequences that, when expressed in a plant, help to increase the resistance of a plant to pathogens. It is recognized that such second nucleotide sequences may be used in either the sense or antisense orientation depending on the desired outcome.

Antisense nucleotides are constructed to hybridize with the corresponding mRNA. Modifications of the antisense sequences may be made as long as the sequences hybridize to and interfere with expression of the corresponding mRNA. In this manner, antisense constructions having 70%, preferably 80%, more preferably 85% sequence identity to the corresponding antisense sequences may be used. Furthermore, portions of the antisense nucleotides may be used to disrupt the

expression of the target gene. Generally, sequences of at least 50 nucleotides, 100 nucleotides, 200 nucleotides, or greater may be used. Examples of such second nucleotide sequences include, but are not limited to, sequences encoding PRI, different members of defensin, or BBE, PR5, antifungal peptides such as tachyplesin, chitinases, glucanase, etc.

Additionally provided are transformed plants, plant cells, plant tissues and seeds thereof.

By "pathogenic agent" are intended pathogenic organisms such as fungi, bacteria, viruses, and disease causing microorganisms. Additionally included are nematodes, insects and the like. Pathogens of the invention include, but are not limited to, viruses or viroids, bacteria, insects, nematodes, fungi, and the like. Viruses include tobacco or cucumber mosaic virus, ringspot virus, necrosis virus, maize dwarf mosaic virus, etc.

Specific fungal and viral pathogens for the major crops include: Soybeans: *Phytophthora megasperma* fsp. *glycinea*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Diaporthe phaseolorum* var. *sojae* (*Phomopsis sojae*), *Diaporthe phaseolorum* var. *caulivora*, *Sclerotium rolfsii*, *Cercospora kikuchii*, *Cercospora sojina*, *Peronospora manshurica*, *Colletotrichum dematium* (*Colletotrichum truncatum*), *Corynespora cassiicola*, *Septoria glycines*, *Phyllosticta sojicola*, *Alternaria alternata*, *Pseudomonas syringae* p.v. *glycinea*, *Xanthomonas campestris* p.v. *phaseoli*, *Microsphaera diffusa*, *Fusarium semitectum*, *Phialophora gregata*, Soybean mosaic virus, *Glomerella glycines*, Tobacco Ring spot virus, Tobacco Streak virus, *Phakopsora pachyrhizi*, *Pythium aphanidermatum*, *Pythium ultimum*, *Pythium debaryanum*, Tomato spotted wilt virus, *Heterodera glycines* *Fusarium solani*; Canola: *Albugo candida*, *Alternaria brassicae*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Mycosphaerella brassiccola*, *Pythium ultimum*, *Peronospora parasitica*, *Fusarium roseum*, *Alternaria alternata*; Alfalfa: *Clavibacter michiganese subsp. insidiosum*, *Pythium ultimum*, *Pythium irregulare*, *Pythium splendens*, *Pythium debaryanum*, *Pythium aphanidermatum*, *Phytophthora megasperma*, *Peronospora trifoliorum*, *Phoma medicaginis* var. *medicaginis*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Leptotrochila medicaginis*, *Fusarium roseum*, *Xanthomonas campestris* p.v. *alfalfae*, *Aphanomyces*

euteiches, *Stemphylium herbarum*, *Stemphylium alfalfae*; Wheat: ***Pseudomonas syringae*** p.v. *atrofaciens*, *Urocystis agropyri*, ***Xanthomonas campestris*** p.v. ***translucens***, *Pseudomonas syringae* p.v. *syringae*, ***Alternaria alternata***, ***Cladosporium herbarum***, ***Fusarium graminearum***, ***Fusarium avenaceum***, ***Fusarium culmorum***, *Ustilago tritici*, *Ascochyta tritici*, ***Cephalosporium gramineum***, *Colletotrichum graminicola*, *Erysiphe graminis* f.sp. ***tritici***, ***Puccinia graminis*** f.sp. *tritici*, *Puccinia recondita* f.sp. *tritici*, ***Puccinia striiformis***, ***Pyrenophora tritici-repentis***, *Septoria nodorum*, ***Septoria tritici***, ***Septoria avenae***, ***Pseudocercosporella herpotrichoides***, *Rhizoctonia solani*, ***Rhizoctonia cerealis***, ***Gaeumannomyces graminis*** var. *tritici*, ***Pythium aphanidermatum***, ***Pythium arrhenomanes***, ***Pythium ultimum***, *Bipolaris sorokiniana*, Barley Yellow Dwarf Virus, Brome Mosaic Virus, Soil Borne Wheat Mosaic Virus, Wheat Streak Mosaic Virus, Wheat Spindle Streak Virus, American Wheat Striate Virus, ***Claviceps purpurea***, ***Tilletia tritici***, ***Tilletia laevis***, ***Ustilago tritici***, ***Tilletia indica***, ***Rhizoctonia solani***, *Pythium arrhenomanes*, ***Pythium gramicola***, ***Pythium aphanidermatum***, High Plains Virus, European wheat striate virus; Sunflower: ***Plasmophora halstedii***, ***Sclerotinia sclerotiorum***, Aster Yellows, ***Septoria helianthi***, *Phomopsis helianthi*, *Alternaria helianthi*, ***Alternaria zinniae***, ***Botrytis cinerea***, *Phoma macdonaldii*, *Macrophomina phaseolina*, *Erysiphe cichoracearum*, *Rhizopus oryzae*, *Rhizopus arrhizus*, ***Rhizopus stolonifer***, ***Puccinia helianthi***, *Verticillium dahliae*, ***Erwinia carotovorum*** pv. ***carotovora***, ***Cephalosporium acremonium***, *Phytophthora cryptogea*, *Albugo tragopogonis*, *Orobanche cumana*; Corn: *Fusarium moniliforme* var. ***subglutinans***, ***Erwinia stewartii***, *Fusarium moniliforme*, *Gibberella zeae (Fusarium graminearum)*, ***Stenocarpella maydi (Diplodia maydis)***, *Pythium irregulare*, ***Pythium debaryanum***, ***Pythium graminicola***, *Pythium splendens*, *Pythium ultimum*, ***Pythium aphanidermatum***, *Aspergillus flavus*, *Bipolaris maydis* O, T *(Cochliobolus heterostrophus)*, *Helminthosporium carbonum* I, II & III *(Cochliobolus carbonum)*, *Exserohilum turcicum* I, II & III, ***Helminthosporium pedicellatum***, ***Physoderma maydis***, *Phyllosticta maydis*, *Kabatie-maydis*, *Cercospora sorghi*, *Ustilago maydis*, *Puccinia sorghi*, *Puccinia polysora*, *Macrophomina phaseolina*, *Penicillium oxalicum*, *Nigrospora oryzae*, *Cladosporium herbarum*, *Curvularia lunata*, *Curvularia inaequalis*, *Curvularia pallescens*, *Clavibacter michiganense*

subsp. *nebraskense*, *Trichoderma viride*, Maize Dwarf Mosaic Virus A & B, Wheat Streak Mosaic Virus, Maize Chlorotic Dwarf Virus, *Claviceps sorghi*, *Pseudomonas avenae*, *Erwinia chrysanthemi* pv. *zea*, *Erwinia corotovora*, *Cornstunt spiroplasma*, *Diplodia macrospora*, *Sclerophthora macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Peronosclerospora maydis*, *Peronosclerospora sacchari*, *Spacelotheca reiliana*, *Physopella zeae*, *Cephalosporium maydis*, *Caphalosporium acremonium*, Maize Chlorotic Mottle Virus, High Plains Virus, Maize Mosaic Virus, Maize Payado Fino Virus, Maize Streak Virus, Maize Stripe Virus, Maize Rough Dwarf Virus; Sorghum: *Exserohilum turcicum*, *Colletotrichum graminicola (Glomerella graminicola)*, *Cercospora sorghi*, *Gloeocercospora sorghi*, *Ascochyta sorghina*, *Pseudomonas syringae* p.v. *syringae*, *Xanthomonas campestris* p.v. *holcicola*, *Pseudomonas andropogonis*, *Puccinia purpurea*, *Macrophomina phaseolina*, *Perconia circinata*, *Fusarium moniliforme*, *Alternaria alternate*, *Bipolaris sorghicola*, *Helminthosporium sorghicola*, *Curvularia lunata*, *Phoma insidiosa*, *Pseudomonas avenae (Pseudomonas alboprecipitans)*, *Ramulispora sorghi*, *Ramulispora sorghicola*, *Phyllachara sacchari*, *Sporisorium reilianum (Sphacelotheca reiliana)*, *Sphacelotheca cruenta*, *Sporisorium sorghi*, Sugarcane mosaic H, Maize Dwarf Mosaic Virus A & B, *Claviceps sorghi*, *Rhizoctonia solani*, *Acremonium strictum*, *Sclerophthora macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Sclerospora graminicola*, *Fusarium graminearum*, *Fusarium oxysporum*, *Pythium arrhenomanes*, *Pythium graminicola*, etc.

Nematodes include parasitic nematodes such as root knot, cyst, reniform and lesion nematodes, etc.

Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc., particularly Coleoptera and Lepidoptera. Insect pests of the invention for the major crops include: Maize: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Helicoverpa zea*, corn earworm; *Spodoptera frugiperda*, fall armyworm; *Diatraea grandiosella*, southwestern corn borer; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Diatraea saccharalis*, surgarcane borer; *Diabrotica virgifera*, western corn rootworm; *Diabrotica longicornis barberi*,

northern corn rootworm; *Diabrotica undecimpunctata howardi*, southern corn rootworm; *Melanotus spp.*, wireworms; *Cyclocephala borealis*, northern masked chafer (white grub); *Cyclocephala immaculata*, southern masked chafer (white grub); *Popillia japonica*, Japanese beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Anuraphis maidiradicis*, corn root aphid; *Blissus leucopterus leucopterus*, chinch bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Hylemya platura*, seedcorn maggot; *Agromyza parvicornis*, corn blot leafminer; *Anaphothrips obscurus*, grass thrips; *Solenopsis milesta*, thief ant; *Tetranychus urticae*, twospotted spider mite; Sorghum: *Chilo partellus*, sorghum borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Feltia subterranea*, granulate cutworm; *Phyllophaga crinita*, white grub; *Eleodes*, *Conoderus*, and *Aeolus spp.*, wireworms; *Oulema melanopus*, cereal leaf beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Sipha flava*, yellow sugarcane aphid; *Blissus leucopterus leucopterus*, chinch bug; *Contarinia sorghicola*, sorghum midge; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Wheat: *Pseudaletia unipunctata*, army worm; *Spodoptera frugiperda*, fall armyworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Agrotis orthogonia*, western cutworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Oulema melanopus*, cereal leaf beetle; *Hypera punctata*, clover leaf weevil; *Diabrotica undecimpunctata howardi*, southern corn rootworm; Russian wheat aphid; *Schizaphis graminum*, greenbug; *Macrosiphum avenae*, English grain aphid; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Mayetiola destructor*, Hessian fly; *Sitodiplosis mosellana*, wheat midge; *Meromyza americana*, wheat stem maggot; *Hylemya coarctata*, wheat bulb fly; *Frankliniella fusca* tobacco thrips; *Cephus cinctus* wheat stem sawfly; *Aceria tulipae*, wheat curl mite; Sunflower: *Suleima helianthana*, sunflower bud moth; *Homoeosoma electellum*, sunflower moth; *zygogramma exclamationis*, sunflower beetle; *Bothyrus gibbosus*, carrot beetle; *Neolasioptera murtfeldtiana*, sunflower seed midge; Cotton: *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton

bollworm; *Spodoptera exigua*, beet armyworm; *Pectinophora gossypiella*, pink bollworm; *Anthonomus grandis*, boll weevil; *Aphis gossypii*, cotton aphid; *Pseudatomoscelis seriatus*, cotton fleahopper; *Trialeurodes abutilonea*, bandedwinged whitefly; *Lygus lineolaris*, tarnished plant bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Thrips tabaci*, onion thrips; *Franklinkiella fusca*, tobacco thrips; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Rice: *Diatraea saccharalis*, sugarcane borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Colaspis brunnea*, grape colaspis; *Lissorhoptrus oryzophilus*, rice water weevil; *Sitophilus oryzae*, rice weevil; *Nephotettix nigropictus*, rice leafhopper *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; Soybean: *Pseudoplusia includens*, soybean looper; Anticarsia gemmatalis, velvetbean caterpillar; *Plathypena scabra*, green cloverworm; *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Spodoptera exigua*, beet armyworm; *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Epilachna varivestis*, Mexican bean beetle; *Myzus persicae*, green peach aphid; *Empoasca fabae*, potato leafhopper; *Acrosternum hilare*, green stink bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Hylemya platura*, seedcorn maggot; *Sericothrips variabilis*, soybean thrips; Thrips tabaci, onion thrips; *Tetranychus turkestani*, strawberry spider mite; *Tetranychus urticae*, twospotted spider mite; Barley: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Schizaphis graminum*, greenbug; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; *Euschistus servus*, brown stink bug; *Delia platura*, seedcorn maggot; *Mayetiola destructor*, Hessian fly; *Petrobia latens*, brown wheat mite; Oil Seed Rape: *Brevicoryne brassicae*, cabbage aphid; *Phyllotreta cruciferae*, Flea beetle; *Mamestra configurata*, Bertha armyworm; *Plutella xylostella*, Diamond-back moth; *Delia* ssp., Root maggots.

The present invention also provides isolated nucleic acids comprising polynucleotides of sufficient length and complementarity to a gene of the invention to use as probes or amplification primers in the detection, quantitation, or isolation of gene transcripts. For example, isolated nucleic acids of the present invention can be used as probes in detecting deficiencies in the level of mRNA in screenings for

desired transgenic plants, for detecting mutations in the gene (e.g., substitutions, deletions, or additions), for monitoring upregulation of expression or changes in enzyme activity in screening assays of compounds, for detection of any number of allelic variations (polymorphisms) of the gene, or for use as molecular markers in plant breeding programs. The isolated nucleic acids of the present invention can also be used for recombinant expression of polypeptides, or for use as immunogens in the preparation and/or screening of antibodies. The isolated nucleic acids of the present invention can also be employed for use in sense or antisense suppression of one or more genes of the invention in a host cell, tissue, or plant. Attachment of chemical agents which bind, intercalate, cleave and/or crosslink to the isolated nucleic acids of the present invention can also be used to modulate transcription or translation. Further, using a primer specific to an insertion sequence (e.g., transposon) and a primer which specifically hybridizes to an isolated nucleic acid of the present invention, one can use nucleic acid amplification to identity insertion sequence inactivated genes of the invention from a cDNA library prepared from insertion sequence mutagenized plants. Progeny seed from the plants comprising the desired inactivated gene can be grown to a plant to study the phenotypic changes characteristic of that inactivation. See *Tools to Determine the Function of Genes* 1995 Proceedings of the Fifteen Annual Corn and Sorghum Industry Research Conference, American Seed Trade Association, Washington, D.C., 1995. Additionally, non-translated 5' or 3' regions of the polynucleotides of the present invention can be used to modulate turnover of heterologous mRNAs and/or protein synthesis. Further, the codon preference characteristic of the polynucleotides of the present invention can be employed in heterologous sequences, or altered in homologous or heterologous sequences to modulate translational level and/or rates.

The present invention provides a method of genotyping a plant comprising a polynucleotide of the present invention. The plant may be a monocot, such as maize or sorghum, or alternatively, a dicot, such as sunflower or soybean. Genotyping provides a means of distinguishing homologues of a chromosome pair and can be used to differentiate segregants in a plant population. Molecular marker methods can be used for phylogenetic studies, characterizing genetic relationships among crop varieties, identifying crosses or somatic hybrids,

localizing chromosomal segments affecting monogenic traits, map based cloning, and the study of quantitative inheritance. See, e.g., *Plant Molecular Biology: A Laboratory Manual*, Chapter 7, Clark, Ed., Springer-Verlag, Berlin (1997). For molecular marker methods, see generally, The DNA Revolution by Andrew H. Paterson 1996 (Chapter 2) in: Genome Mapping in Plants (ed. Andrew H. Paterson) by Academic Press/R. G. Landis Company, Austin, Texas, pp. 7-21.

The particular method of genotyping in the present invention may employ any number of molecular marker analytic techniques such as, but not limited to, restriction fragment length polymorphisms (RFLPs). Thus, the present invention further provides a means to follow segregation of a gene or nucleic acid of the present invention as well as chromosomal sequences genetically linked to these genes or nucleic acids using such techniques as RFLP analysis. Linked chromosomal sequences are within 50 centiMorgans (cM), often within 40 or 30 cM, preferably within 20 or 10 cM, more preferably within 5, 3, 2, or 1 cM of a gene of the invention.

In the present invention, the nucleic acid probes employed for molecular marker mapping of plant nuclear genomes selectively hybridize, under selective hybridization conditions, to a gene encoding a polynucleotide of the present invention. In preferred embodiments, the probes are selected from polynucleotides of the present invention. Typically, these probes are cDNA probes or *Pst I* genomic clones. The length of the probes is discussed in greater detail, *supra*, but are typically at least 15 bases in length, more preferably at least 20, 25, 30, 35, 40, or 50 bases in length. Generally, however, the probes are less than about 1 kilobase in length. Preferably, the probes are single copy probes that hybridize to a unique locus in a haploid chromosome complement.

The present invention further provides a method of genotyping comprising the steps of contacting, under stringent hybridization conditions, a sample suspected of comprising a polynucleotide of the present invention with a nucleic acid probe. Generally, the sample is a plant sample; preferably, a sample suspected of comprising a maize polynucleotide of the present invention (e.g., gene, mRNA). The nucleic acid probe selectively hybridizes, under stringent conditions, to a subsequence of a polynucleotide of the present invention comprising a polymorphic marker. Selective hybridization of the nucleic acid

probe to the polymorphic marker nucleic acid sequence yields a hybridization complex. Detection of the hybridization complex indicates the presence of that polymorphic marker in the sample. In preferred embodiments, the nucleic acid probe comprises a polynucleotide of the present invention.

Methods are provided for controlling plant pathogens comprising applying an anti-pathogenic amount of a protein or composition of the invention to the environment of the pathogens. By "controlling plant pathogens" is intended killing the pathogen or preventing or limiting disease formation on a plant. By "anti-pathogenic amount" is intended an amount of a protein or composition that controls a pathogen. The proteins and compositions can be applied to the environment of the pathogen by methods known to those of ordinary skill in the art.

The proteins of the invention can be formulated with an acceptable carrier into a pesticidal composition(s) that is for example, a suspension, a solution, an emulsion, a dusting powder, a dispersible granule, a wettable powder, and an emulsifiable concentrate, an aerosol, an impregnated granule, an adjuvant, a coatable paste, and also encapsulations in for example, polymer substances.

Such compositions disclosed above may be obtained by the addition of a surface-active agent, an inert carrier, a preservative, a humectant, a feeding stimulant, an attractant, an encapsulating agent, a binder, an emulsifier, a dye, a U.V protectant, a buffer a flow agent or fertilizers, micronutrient donors or other preparations that influence plant growth. One or more agrochemicals including, but not limited to, herbicides, insecticides, fungicides, bacteriocides, nematocides, molluscicides, acaricides, plant growth regulators, harvest aids and fertilizers, can be combined with carriers, surfactants or adjuvants customarily employed in the art of formulation or other components to facilitate product handling and application for particular target pests. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders or fertilizers. The active ingredients of the present invention are normally applied in the form of compositions and can be applied to the crop area or plant to be treated, simultaneously or in succession, with other compounds. Preferred methods of applying an active ingredient of the present

invention or an agrochemical composition of the present invention, which contains at least one of the proteins of the present invention, are foliar application, seed coating and soil application. The number of applications and the rate of application depend on the intensity of infestation by the corresponding pest.

Suitable surface-active agents include, but are not limited to, anionic compounds such as a carboxylate of, for example, a metal; carboxylate of a long chain fatty acid; an N-acylsarcosinate; mono or di-esters of phosphoric acid with fatty alcohol ethoxylates or salts of such esters; fatty alcohol sulfates such as sodium dodecyl sulfate, sodium octadecyl sulfate or sodium cetyl sulfate; ethoxylated fatty alcohol sulfates; ethoxylated alkylphenol sulfates; lignin sulfonates; petroleum sulfonates; alkyl aryl sulfonates such as alkyl-benzene sulfonates or lower alkylnaphtalene sulfonates, e.g. butyl-naphthalene sulfonate; salts of sulfonated naphthalene-formaldehyde condensates; salts of sulfonated phenol-formaldehyde condensates; more complex sulfonates such as the amide sulfonates, e.g. the sulfonated condensation product of oleic acid and N-methyl taurine; or the dialkyl sulfosuccinates, e.g. the sodium sulfonate or dioctyl succinate. Non-ionic agents include condensation products of fatty acid esters, fatty alcohols, fatty acid amides or fatty-alkyl- or alkenyl-substituted phenols with ethylene oxide, fatty esters of polyhydric alcohol ethers, e.g. sorbitan fatty acid esters, condensation products of such esters with ethylene oxide, e.g. polyoxyethylene sorbitar fatty acid esters, block copolymers of ethylene oxide and propylene oxide, acetylenic glycols such as 2, 4, 7, 9-tetraethyl-5-decyn-4, 7-diol, or ethoxylated acetylenic glycols. Examples of a cationic surface-active agent include, for instance, an aliphatic mono-, di-, or polyamine such as an acetate, naphthenate or oleate; or oxygen-containing amine such as an amine oxide of polyoxyethylene alkylamine; an amide-linked amine prepared by the condensation of a carboxylic acid with a di- or polyamine; or a quaternary ammonium salt.

Examples of inert materials include but are not limited to inorganic minerals such as kaolin, phyllosilicates, carbonates, sulfates, phosphates or botanical materials such as cork, powdered corncobs, peanut hulls, rice hulls, and walnut shells.

The compositions of the present invention can be in a suitable form for direct application or as concentrate of primary composition, which requires

dilution with a suitable quantity of water or other diluent before application. The pesticidal concentration will vary depending upon the nature of the particular formulation, specifically, whether it is a concentrate or to be used directly. The composition contains 1 to 98% of a solid or liquid inert carrier, and 0 to 50%, preferably 0.1 to 50% of a surfactant. These compositions will be administered at the labeled rate for the commercial product, preferably about 0.01 lb-5.0 lb. per acre when in dry form and at about 0.01 pts. - 10 pts. per acre when in liquid form.

In a further embodiment, the compositions, as well as the proteins of the present invention can be treated prior to formulation to prolong the activity when applied to the environment of a target pest as long as the pretreatment is not deleterious to the activity. Such treatment can be by chemical and/or physical means as long as the treatment does not deleteriously affect the properties of the composition(s). Examples of chemical reagents include but are not limited to halogenating agents; aldehydes such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride; alcohols, such as isopropanol and ethanol; and histological fixatives, such as Bouin's fixative and Helly's fixative (see, for example, Humason, Animal Tissue Techniques, W H. Freeman and Co., 1967).

The compositions can be applied to the environment of a pest by, for example, spraying, atomizing, dusting, scattering, coating or pouring, introducing into or on the soil, introducing into irrigation water, by seed treatment or general application or dusting at the time when the pest has begun to appear or before the appearance of pests as a protective measure. It is generally important to obtain good control of pests in the early stages of plant growth, as this is the time when the plant can be most severely damaged. The compositions of the invention can conveniently contain another insecticide or pesticide if this is thought necessary.

Plants to be protected within the scope of the present invention include but are not limited to cereals (wheat, barley, rye, oats, rice, sorghum and related crops), beets (sugar beet and fodder beet), drupes, pomes and soft fruit (apples, pears, plums, peaches, almonds, cherries, strawberries, raspberries, and blackberries), leguminous plants (alfalfa, beans, peanuts, lentils, peas, soybeans), oil plants (rape, mustard, poppy, olives, safflowers, sunflowers, coconuts, castor oil plants, cocoa beans, oil palms), cucumber plants (cucumber, marrows, melons), fiber plants (cotton, flax, hemp, jute), citrus fruit (oranges, lemons, limes, grapefruit,

mandarins), vegetables (spinach, lettuce, asparagus, cabbages and other *Brassicae*, carrots, onions, tomatoes, potatoes, paprika), *lauraceae* (avocados, cinnamon, camphor), deciduous trees and conifers (e.g. linden-trees, yew-trees, oak-trees, alders, poplars, birch-trees, firs, larches, pines), or plants such as maize, turf plants, tobacco, nuts, coffee, sugar cane, tea, hops, bananas and natural rubber plants, as well as ornamentals

In a further embodiment, formulations of the present invention for use as antimicrobial therapeutics comprise the anti-pathogenic proteins in a physiologically or pharmaceutically acceptable carrier, such as an aqueous carrier. Thus, formulations for use in the present invention include, but are not limited to, those suitable for parenteral administration including subcutaneous, intradermal, intramuscular, intravenous and intraarterial administration, as well as topical administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art. Such formulations are described in, for example, *Remington's Pharmaceutical Sciences* 19th ed., Gosl[illegible], Mack Easton PA (1980).

In the manufacture of a medicament according to the invention, the anti-pathogenic compositions are typically admixed with, inter alia, an acceptable carrier. The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious or harmful to the patient. The carrier may be a solid or a liquid. One or more anti-pathogenic proteins may be incorporated in the formulations of the invention, which may be prepared by any of the well-known techniques of pharmacy consisting essentially of admixing the components, optionally including one or more accessory therapeutic ingredients.

Formulations of the present invention may comprise sterile aqueous and non-aqueous injection solutions of the active compound, which preparations are preferably isotonic with the blood of intended recipient and essentially pyrogen free. These preparations may contain anti-oxidants, buffers, bacteriostats and solutes that render the formulation isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored

in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

In the formulation the anti-pathogenic protein may be contained within a lipid particle or vesicle, such as a liposome or microcrystal, which may be suitable for parenteral administration. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the targeted cassette is contained therein. Positively charged lipids such as N-[1-(2,3-dioleoyloxi)propyl]-N,N,N-trimethyl-amoniummetylsulfate, or "DOTAP", are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well-known. See, e.g., U.S. Patent Nos. 4,880,635 to Janoff *et al.*; 4,906,477 to Kurono *et al.*; 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen *et al.*; 4,921,757 to Wheatley *et al.*; etc.

The dosage of the anti-pathogenic protein administered will vary with the particular method of administration, the condition of the subject, the weight, age, and sex of the subject, the particular formulation, the route of administration, etc. In general, the protein will be administered in a range of about 1μg/L to about 10g/L.

The following examples are offered by way of illustration and not by way of limitation

EXPERIMENTAL

Materials and Methods

Plant material

Sunflower plants were grown in the greenhouse and growth chamber. The sunflower line SMF 3 and oxox-transgenic sunflower (line 193870 and 610255) were used for RNA profiling study by CuraGen using methods described in U.S. Patent No. 5,871,697 to Rothberg *et al.*, and U.S. Patent No. 5,972,693 to Rothberg *et al.*, both incorporated herein by reference. Sunflower pathogen, *Sclerotinia sclerotiorum* was maintained on plate at 20°C in dark.

Preparation of total RNAs for RNA profiling study and Northern analysis

Plant materials were ground in liquid nitrogen, and total RNA was extracted by the Tri-Reagent Method (Sigma). For each RNA profiling study, RNA

samples from 6-week-old sunflower leaves and stems of transgenic sunflower plants expressing a wheat oxalate oxidase gene were compared with those from sunflower line SMF3. Total RNA (20 μg) was separated in a 1% agarose gel containing formaldehye. Ethidium bromide was included to verify equal loading of RNA. After transfer onto Hybond N+ membrane (Amersham), the blots were hybridized with ^{32}P-labelled PR5, defensin or BBE cDNA probes. A duplicate blot was hybridized with an 18S rRNA probe as a control. Hybridization and washing conditions were performed according to Church *et al.* (1984) *Proc. Natl. Acad. Sci. USA 81*:1991-1995.

RNA profiling technology

Total RNA was analyzed using the gene expression profiling process (GeneCalling®) as described in U.S. Patent No. 5,871,697, herein incorporated by reference. A number of distinct transcripts increased in abundance following the oxidative burst and cDNAs corresponding to a portion of these transcripts were cloned and sequenced.

Isolation of full-length or flanking sequences by PCR amplification of cDNA ends

Three defense-related cDNAs were isolated by using RNA profiling and PCR-based technologies. RNA profiling studies were conducted through the collaboration with CuraGen Corporation. Figure 1 illustrates the cloning strategy used. The sequence information generated was used for designing gene-specific primers to amplify both 3′ and/or 5′ end regions of the target genes using the PCR-based, RACE method. *Sclerotinia*-infected and oxox-induced cDNA libraries or cDNAs made using a Marathon cDNA Amplification Kit (Clontech) were utilized as a source of templates for PCR amplification. To facilitate cloning full-length cDNAs from the initially cloned regions, we designed a pair of 28 bp vector primers flanking cDNAs on the both ends (3′ and 5′) of the pBS vector and directionally amplified either the 5′ or 3′ end of a cDNA with one of vector primers (pBS-upper or pBS-lower) and a gene-specific primer. Once the anticipated 5′ end of a specific gene with an intact ATG start codon was cloned and sequenced, the full-length cDNA was amplified using a second gene-specific primer containing

corresponding to sequence upstream of the ATG and a vector primer at 3' end. The PCR products were cloned and sequenced by standard methods.

PCR reactions were performed in a total volume of 25 µl in 10 mM Tris-HCl, pH 8.3; 1.5 mM $MgCl_2$; 50 mM KCl; 0.1 mM dNTPs; 0.25 µM of each primer with 0.5 units of advantage cDNA polymerase mix (Clontech) or Pwo DNA polymerase (Boehringer). Genomic DNA and/or cDNA library mixtures were used as a source of templates for PCR amplification.

Isolation of pathogen-inducible promoters

Promoter regions of PR5, defensin, and BBE were isolated from sunflower genomic DNA using Universal GenomeWalker Kit (Clontech) according to the manufacturer's instructions. Restriction digested genomic DNAs were ligated with an adapter to construct pools of genomic DNA fragments for walking by PCR (Siebert *et al.* (1995) *Nuc. Acids Res.* 23:1087-1088).

Analysis of amplified PCR products

Amplified PCR fragments with the expected sizes were individually sliced out of a gel for a second round of PCR amplification with the same conditions as the initial PCR. Each second-round PCR product yielding a single band of the expected size was cloned into a TA vector (Clontech) according to the manufacturer's instructions. Identified positive clones were selected for DNA sequencing using an Applied BioSystems 373A (ABI) automated sequencer at the Nucleic Acid Analysis Facility of Pioneer Hi-Bred International, Incorporated. DNA sequence analysis was carried out with the Sequencer (3.0). Multiple-sequence alignments (Clustal W) of the DNA sequence were analyzed with the Curatool (CuraGen).

Construction of the Sclerotinia-infected and resistance-enhanced (oxox-induced) sunflower cDNA libraries

Six-week-old SMF3 sunflower plants were infected with *Sclerotinia sclerotiurn* by petiole inoculation with *Sclerotinia*-infested carrot plugs. Six days after infection, leaf and stem tissues were collected from infected plants for total RNA isolation. Total RNA was also isolated from sunflower oxox-transgenic

plants (line 610255) expressing a wheat oxalate oxidase gene at the six-week stage. Previous studies have shown that elevated levels of H_2O_2, SA, and PR1 protein were deducted in oxox-transgenic plants at six-week stage and the plants showed more resistance to *Sclerotinia* infection (WO 99/04013). The mRNAs were isolated using an mRNA purification kit (BRL) according to manufacturer's instruction. cDNA libraries were constructed with the ZAP-cDNA synthesis kit into pBluescrip phagemid (Stratagene). A cDNA library mixture for PCR cloning was made of oxox transgenic stem and Sclerotinia-infected leaf libraries (1:2 mix).

Fungal infection and chemical treatments

Sunflower plants SMF3 were planted in 4-inch pots and grown in the greenhouse for four weeks. After transfer to the growth chamber, plants were maintained under 12 hour photoperiod at 22°C with a 80% relative humidity. Six-week-old plants were inoculated with *Sclerotinia*-infested carrot plugs or sprayed with one of four different chemical treatments. For each plant, three petioles were inoculated and wrapped with 1x2 inch parafilm. Plant tissue samples were collected at different time points by immediately freezing in liquid nitrogen and then stored at -80°C.

Results

RNA profiling study of oxox-transgenic sunflower plants

Resistance to the fungal pathogen *Sclerotinia* is a trait of major importance for crops such as sunflower, canola, and soybean. Sunflower *Sclerotinia* disease can be established at various developmental stages with the main targets being head, stem, and root tissues. This suggests that resistance genes need to be constitutively expressed in multiple tissues. The major toxic and pathogenic factor produced by *Sclerotinia* is oxalic acid that can be converted into H_2O_2 and CO_2 by oxalate oxidase. A candidate gene for detoxifying oxalate is the wheat oxalate oxidase (oxox) which have been used to transform a sunflower inbred line. Expression of oxox by a constitutive promoter significantly enhances resistance to *Sclerotinia* in sunflower. In a growth chamber experiment, lesion size was six-fold lower in oxox-transgenic sunflower plants upon infection with *Sclerotinia* mycelia relative to untransformed plants. At the six-week-old stage, the oxox-transgenic

sunflower plants displayed a lesion mimic in the mature leaves. The enhanced *Sclerotinia* resistance of sunflower oxox transgenics is closely related to the observed elevated levels of SA and PR proteins (WO 99/04013).

In the RNA profiling analysis, 30 bands were induced and 30 bands were repressed in the oxox-transgenic stem and leaf tissues compared to non-transformed SMF3 plants. Three of the induced bands were sequenced (Table1), and the sequence information was used to clone the full-length clones.

Cloning of full-length cDNAs related to sunflower disease resistance

A PCR-based cloning method was developed to efficiently isolate full-length cDNAs of the plant defense genes, from sunflower cDNA libraries (Figure 2). A cDNA library mixture containing both oxox-transgenic cDNA library and Sclerotinia-infected cDNA library (1:2 ratio) was used as template for PCR amplification. Using cDNA libraries as DNA template in PCR amplification had two benefits: (1) the number of unexpected PCR products was reduced as compared to genomic DNA as a source of template, and (2) disease-induced cDNA libraries increased the chance of isolating defense-related genes. To facilitate cloning full-length cDNAs from the initial cloned regions, we designed a pair of 28 bp vector primers (Table 1) flanking cDNAs on the both ends (3′ and 5′) of the vector and directionally amplified either the 5′ or 3′ end of a cDNA with one vector primer and a gene-specific primer (Figure 1 and Table 1). The anticipated 5' end of specific gene with the intact ATG start codon was cloned and sequenced. The full-length cDNA was amplified using a second gene-specific primer containing sequence upstream of the ATG and a vector primer at the 3′ end. The PCR products were cloned and submitted to sequence analysis.

Table 1 provides RNA profiling band sequences (PBS) and oligonucleotide sequences used for PCR amplification of the cDNAs and promoter regions. Oligonucleotide PBS-upper (P3) and PBS-lower (P4) were two primers located at the ends of cDNA library vector, as indicated in Figure 2. For each targeted gene, two or three gene-specific primers were made to complete the 5′- end RACE (P1), the 3′- end RACE (P2), and the full-length RACE (P5). The additional antisense primers were made for cloning promoter regions of PR5, defensin, and BBE, using

the GenomeWalker kit (Clontech) (Band h0a0-231.3, PR5; band d0l0-113.9, defensin; and n0s0-152.7, BBE).

Table 1: Oligonucleotide sequences used for PCR amplification of cDNAs and promoter regions:

cDNA cloning:
Library vector (pBS):
PBS-upper: GCGATTAAGTTGGGTAACGCCAGGGT (SEQ ID NO:26)
PBS-lower: TCCGGCTCGTATGTTGTGTGGAATTG (SEQ ID NO:27)

PR5:
h0a0-231.3:
TGATCAGTTTTGTACACGGTGCAAGGGTTATTGCACCCGCCAGA
GCCCGTAACTCNCCAGGACACTGGCCATTGATATCCGCAGTACA
TGAGATACCCCGGGTGCACCCATTAGAATTGGGTCTAAACACCA
TCGGCACATTGAATCCGTCCACAAGAGAAATGTCAAAGAAATCA
AGATTGTTGAACTGGTTCCAAGCGTACTCGGCCCATGTGTTTGG
GTGGGGTACC (SEQ ID NO:28)

Sense: CCGAGTACGCTTTAACCAGT (SEQ ID NO:29)

Antisense: TCCGCAGTACATGAGATACCC (SEQ ID NO: 30)
Full RACE: ACAATGACAACCTCCACCCTTCCCACTTT (SEQ ID NO:31)
(P5

Defensin:
d0l0-113.9:
TCCGGACCATGTCTGGCTTGCCTTCTCACATAATTCTCCTTTCAC
CGATCCGATTTCTGAGATAGCAAGAACAAAGAGAAGCAGAAGA
AAAGCATTGAAAGCAACTGAAATT (SEQ ID NO:32)

A-sense: GACCATGTCTGGCTTGCCTTCTCACA (SEQ ID NO:33)
full-RACE: GAGCTTGAGCTTAGTTCAGTAACTTAAAAATGGCC (SEQ ID NO:34)
(P5)

BBE:
n0s0-152.7:
TCTACACATTTGGTGCGAAGATGGAGGAGTACTCAGATACAGCA
ATTTCGTATCCCCATAGAGCTGGGGTGTTGTACCAAGTGTTCAA
GAGGTGGACTTCGTCCATCAGCCTTCGGACAAGACCTTGATAT
CACTCAGACGGTTGGCTTGGCTCCGAAGCTT (SEQ ID NO: 35)

Sense: CCAACCGTCTGAGTGATATCAAGG (SEQ ID NO:36)
A-sense: GGGAAGATGGAGGAGTACTCAGAT (SEQ ID NO:37)
Full-RACE: CGGCACGAGTAACTCTCGTTCAGTGTTCC (SEQ ID NO:38)
(P5)

Promoter cloning:
AP Primer: GTAATACGACTCACTATAGGGC (SEQ ID NO:39)
PR5 A-sense2: CGAATAGTGAACACGGCTGCATTGGT (SEQ ID NO:40)
BBE A-sense2: GCTGCAGCTTGCCAAATGGGTATGTA (SEQ ID NO:41)

* Oligonucleotide PBS-upper (P3) and PBS-lower (P4) were two primers located at the ends of cDNA library vector, as indicated in Figure 2. For each targeted gene, two or three gene specific primers were made to complete the 5' end RACE (P1), the 3' end RACE (P2), and the full-length RACE (P5). The additional antisense primers were made for cloning promoter regions of PR5-1 and BBE, using the genome walker kit from Clontech. Band h0a0-231.3, PR5-1; band d010-113.9, defensin; and band s0-162.7, BBE

Cloning sunflower PR5-1 cDNA and its promoter

A full-length cDNA encoding pathogenesis-related protein-5 (PR5-1) was isolated from sunflower. The nucleotide sequence of PR5-1 is set forth in SEQ ID:4 and the amino acid sequence encoded by this nucleotide sequence is set forth in SEQ ID:1. The sunflower PR5-1 protein with its amino-terminal signal sequence is 233 amino acids in length with a calculated molecular mass of 25 kDa and a pI of 6.71. Database searches with predicted amino acid sequence revealed significant sequence similarity with previously reported PR5 proteins from other plant species. The 5'-flanking sequence of the PR5-1 gene contains two potential pathogen-responsive MRE-like elements. These elements have the sequences TGTAGG (nucleotides 23-28, SEQ ID:7) and AACAAAA (nucleotides 247-253, SEQ ID:7). The PR5-1 promoter region also contains a CAAT box (nucleotides 438-441, SEQ ID:7) and a TATA box (nucleotides 485-490, SEQ ID:7). Figure 2 shows the alignment of amino acid sequence of PR5-1 from sunflower with other PR5 or osmotin-like proteins from grape, soybean, tomato, and potato. Sunflower PR5-1 shows the highest sequence similarity to P21 protein (78% amino acid identity;

80% similarity) from soybean (Swiss-Prot P205096) followed by the osmotin-like protein from grape (Swiss-Prot O04708 ; 72% amino acid identity; 77% similarity), where sequence comparisons were performed with the GAP algorithm described above using default parameters.

Berberine Bridge Enzyme (BBE) cDNA and its promoter

A full-length cDNA encoding a BBE homologue was isolated from sunflower. The full-length cDNA set forth in SEQ ID:5 is 1809 nucleotides long with an open reading frame encoding a protein of 542 amino acids (SEQ ID:2) and a calculated molecular mass at 61.41 kDa and a pI of 8.18 (Figure 5). The BBE promoter region contains a potential MRE-like element with the sequence TGTAGC (nucleotides 139-144, SEQ ID:8). The BBE promoter also contains a CAAT box (nucleotides 278-281, SEQ ID:8), and a TATA box (nucleotides 485-490, SEQ ID:8). The isolated cDNA shares homology with BBE cDNAs from California poppy and opium poppy (Figure 3) and two published sunflower cDNA's encoding carbohydrate oxidases (WO 98/13478), which have antifungal activity, specifically against *Phytophthora* and *Pythium* species (Figure 3). The amino acid sequence alignment indicates 42% identity and 52% similarity between the sunflower BBE and the previously patented sequences (Sunflower-15 and Sunflower-17 from WO 98/13478), where the comparison was performed with the GAP algorithm described above using the default parameters.

Inducible sunflower defensin cDNA and its promoter

The sunflower defensin cDNA is 556 nucleotides long with an open reading frame starting at nucleotide 36 and ending at nucleotide position 362 (SEQ ID:6). The deduced polypeptide is 108 amino acids long and contains a putative signal peptide at the amino-terminal end (SEQ ID:3). The cloned defensin promoter contains two W-boxes with the nucleotide sequence TTGACC (nucleotides 221-226, and nucleotides 1075-1080, SEQ ID:9), and a G-box with sequence CACGTG (nucleotides 554-569, SEQ ID:9). These cis-elements are potentially related to plant defense response. The defensin promoter also contains a TATA box (nucleotides 857-860, SEQ ID:9).The protein has significant homology to other

reported plant defensins (Figure 4). Eight important cysteine residues in this novel defensin were highly conserved among all other known plant defensins.

Accumulation of PR5-1, defensin and BBE transcripts in response to fungal pathogen infection and chemical treatments

The expression of many of PR5 and defensin genes were induced by biotic and abiotic stresses (Terra *et al.* (1988) *Planta 206*:117-124); Ward *et al.* (1991) *Plant Cell* 3:1085-1094). Oxalic acid (OA), a compound produced by Sclerotinia and many other fungal pathogens *in planta*, plays an important role in the disease infection process (Noyes *et al.* (1981) *Physiol. Plant Path. 18*:123-132). Salicylic acid, jasmonic acid and H_2O_2 have been implicated as having a central role in plant disease resistance and systemic acquired resistance, and have been shown to induce the accumulation of many PR proteins including PR5 protein and defensin in *Arabidopsis* (Blechert *et al.* (1995) *Proc. Natl. Acad. Sci. USA 92*:4099-4105; Terra *et al.* (1988) *Planta 206*:117-124; Noyes *et al.* (1981) *Physiol. Plant Path. 18*:123-132)

Six-week-old sunflower plants were either inoculated with Sclerotinia or treated with different chemicals. Plants inoculated with Sclerotinia showed wilt symptoms on inoculated leaves 24 hours after inoculation and lesions started to spread to the main stem 3 days after infection. For the infection experiment, plant tissues were collected at 0, 6, 12, 24 hours, and 3, 6 and 10 days after infection. Chemical-treated plants were collected at 0, 6, 12, and 24 hours after foliar application.

Northern blot analysis revealed that sunflower PR5-1 protein was induced in leaf and stem tissues of the *Sclerotinia*-infected and oxox transgenic plants. RNA profiling indicated that PR5-1 transcript level in the oxox transgenic plants was 9-fold higher than in the untransformed line (SMF3). Northern results indicated that the sunflower PR5-1 was up-regulated significantly by Jasmonic acid (450 μM) and oxalic acid (5 mM). Up-regulation was less pronounced between control and salicylic acid, and H_2O_2 treated samples.

BBE transcripts were highly induced in oxox-transgenic and *Sclerotinia* infected sunflower leaves. However, BBE transcripts were not detected in either control or infected stem samples. Northern blot analysis confirmed the RNA

profiling result of increased BBE transcripts in oxox transgenic plants. The chemical induction experiment revealed that BBE expression was induced by oxalic acid, H_2O_2, SA and JA at early time points and returned to the normal level within 24 hours after application.

The expression of the isolated sunflower defensin gene appeared to be different from other defensin genes. In general, plant defensin genes such as *Arabidopsis* PDF1.2 and radish defensin are induced by pathogens via an SA-independent and JA-dependent pathway. Northern results indicated that the sunflower defensin was up-regulated significantly by salicylic acid (5 mM), oxalic acid (5 mM) and H_2O_2 (5 mM). However, there was little difference between control and jasmonic acid treated samples.

Defensin transcript levels were significantly higher in samples from oxox transgenic plants relative to levels in control plants. Northern analysis revealed that sunflower defensin was induced in leaf tissue of the Sclerotinia-infected and oxox transgenic plants. A time course study showed that defensin, PR5-1 and BBE transcripts were highly induced in oxox-transgenic tissues at the 6-week-old stage. These results indicate that the defense pathways were activated in oxox transgenic sunflowers at this stage.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

Applicant's or agent's file reference	5718-99	International application No.	PCT/US00/

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 5, lines 23,28,33; page 6, line 5; page 58, line 25; page 57, line 13; page 58, line 14; page 59, line 13 and page 60, line 12.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depository institution
American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Blvd.
Manassas, VA 20110-2209 US

Date of deposit	Accession Number
13 May 1999 (13.05.99)	PTA-73

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

PTA-67 - deposited with ATCC on 13.05.99; page 5, lines 23, 28; page 6, line 4, 5; page 55, line 11; page 56, line 31; page 57, line 33; page 58, line 32; page 59, lines 10, 13; PTA-75 deposited with ATCC on 13.05.99; page 5, lines 24, 29; page 6, lines 1, 5; page 55, line 18; page 57, line 6; page 58, line 7; page 59, line 8; page 60, line 9 - PTA-76, deposited with ATCC on 13.05.99; page 6, lines 3, 5 - PTA-561, deposited with ATCC ON 31.08.99; page 5, line 25; page 6, lines 2, 5; page 60, line 27

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indicators are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application	☐ This sheet was received with the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July 1998)

THAT WHICH IS CLAIMED:

1. A method for increasing pathogen resistance in a plant, said method comprising transforming said plant with a DNA construct comprising a nucleotide sequence selected from the group consisting of:

a) sunflower PR5 homologue;

b) a nucleotide sequence encoding the amino acid sequence of SEQ ID:1;

c) the nucleotide sequence set forth in SEQ ID:4;

d) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:4;

e) a nucleotide sequence deposited as Patent Deposit No. PTA-67;

f) a sunflower defensin homologue;

g) a nucleotide sequence encoding the amino acid sequence of SEQ ID:3;

h) the nucleotide sequence set forth in SEQ ID: 6;

i) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:6;

j) a nucleotide sequence deposited as Patent Deposit No. PTA-75;

k) a sunflower BBE homologue;

l) a nucleotide sequence encoding the amino acid sequence of SEQ ID:2;

m) the nucleotide sequence set forth in SEQ ID:5;

n) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:5.

o) a nucleotide sequence deposited as Patent Deposit No. PTA-73;

p) a nucleotide sequence that hybridizes to the sequence of any one of a)-o) under stringent conditions;

wherein said nucleotide sequence is operably linked to a promoter that drives expression of a coding sequence in a plant cell and regenerating stably transformed plants.

2. The method of claim 1, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% sodium dodecyl sulphate at 37° C, and a wash in 15 mM NaCl, 1.5 mM trisodium citrate at 60°C.

3. The method of claim 1, wherein said pathogen is a fungal pathogen.

4. The method of claim 1, wherein said plant is a dicot.

5. The method of claim 1, wherein said plant is a monocot.

6. The method of claim 1, wherein said promoter is a constitutive promoter.

7. The method of claim 6, wherein said constitutive promoter is selected from the scp1 or ucp promoter.

8. The method of claim 1, wherein said promoter is an inducible promoter.

9. The method of claim 8, wherein said promoter is a pathogen-inducible promoter.

10. A plant having stably incorporated into its genome a DNA construct comprising a nucleotide sequence selected from the group consisting of:

a) a sunflower PR5 homologue;

b) a nucleotide sequence encoding the amino acid sequence of SEQ ID:1;

c) the nucleotide sequence set forth in SEQ ID:4;

d) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:4;

e) a nucleotide sequence deposited as Patent Deposit No. PTA-67;

f) a sunflower defensin homologue;

g) a nucleotide sequence encoding the amino acid sequence of SEQ ID:3;

h) the nucleotide sequence set forth in SEQ ID:6;

i) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:6;

j) a nucleotide sequence deposited as Patent Deposit No. PTA-75;

k) a sunflower BBE homologue;

l) a nucleotide sequence encoding the amino acid sequence of SEQ ID:2;

m) the nucleotide sequence set forth in SEQ ID:5;

n) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:5;

o) a nucleotide sequence deposited as Patent Deposit No. PTA-73;

p) a nucleotide sequence that hybridizes to the sequence of any one of a)-o) under stringent conditions;

wherein said nucleotide sequence is operably linked to a promoter that drives expression of a coding sequence in a plant cell.

11. The plant of claim 10, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% sodium dodecyl sulphate at 37° C, and a wash in 15 mM NaCl, 1.5 mM trisodium citrate at 60°C.

12. Seed of the plant according to claim 10.

13. A plant cell having stably incorporated into its genome a DNA construct comprising a nucleotide sequence selected from the group consisting of:

a) a sunflower PR5 homologue;

b) a nucleotide sequence encoding the amino acid sequence of SEQ ID:1;

c) the nucleotide sequence set forth in SEQ ID:4;

d) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:4;

e) a nucleotide sequence deposited as Patent Deposit No. PTA-67;

f) a sunflower defensin homologue;

g) a nucleotide sequence encoding the amino acid sequence of SEQ ID:3;

h) the nucleotide sequence set forth in SEQ ID:6;

i) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:6;

j) a nucleotide sequence deposited as Patent Deposit No. PTA-75;

k) a sunflower BBE homologue;

l) a nucleotide sequence encoding the amino acid sequence of SEQ ID:2;

m) the nucleotide sequence set forth in SEQ ID:5;

n) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:5;

o) a nucleotide sequence deposited as Patent Deposit No. PTA-73;

p) a nucleotide sequence that hybridizes to the sequence of any one of a)-o) under stringent conditions;

wherein said nucleotide sequence is operably linked to a promoter that drives expression of a coding sequence in a plant cell.

14. The plant cell of claim 13, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% sodium dodecyl sulphate at 37° C, and a wash in 15 mM NaCl, 1.5 mM trisodium citrate at 60°C.

15. An isolated nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

a) a sunflower PR5 homologue;

b) a nucleotide sequence encoding the amino acid sequence of SEQ ID:1;

c) the nucleotide sequence set forth in SEQ ID:4;

d) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:4;

e) a nucleotide sequence deposited as Patent Deposit No. PTA-67;

f) a sunflower defensin homologue;

g) a nucleotide sequence encoding the amino acid sequence of SEQ ID:3;

h) the nucleotide sequence set forth in SEQ ID:6;

i) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:6;

j) a nucleotide sequence deposited as Patent Deposit No. PTA-75;

k) a sunflower BBE homologue;

l) a nucleotide sequence encoding the amino acid sequence of SEQ ID:2:

m) the nucleotide sequence set forth in SEQ ID:5;

n) a nucleotide sequence that shares at least 60% identity to the sequence of SEQ ID:5;

o) a nucleotide sequence deposited as Patent Deposit No. PTA-73;

p) a nucleotide sequence that hybridizes to the sequence of any one of a)-o) under stringent conditions.

16. The nucleic acid molecule of claim 15, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% sodium dodecyl sulphate at 37° C. and a wash in 15 mM NaCl, 1.5 mM trisodium citrate at 60°C.

17. A DNA construct comprising a nucleotide sequence of claim 15.

18. A vector comprising a nucleotide sequence of claim 17.

19. A substantially purified protein molecule having an amino acid sequence selected from the group consisting of:

a) a sunflower PR5;

b) the amino acid sequence set forth in SEQ ID:1;

c) an amino acid sequence that shares at least 60% sequence similarity to the sequence of SEQ ID:1;

d) an amino acid sequence encoded by the nucleotide sequence deposited as Patent Deposit No. PTA-67;

e) a sunflower defensin;

f) the amino acid sequence set forth in SEQ ID:3;

g) an amino acid sequence that shares at least 60% sequence similarity to the sequence of SEQ ID:3;

h) an amino acid sequence encoded by the nucleotide sequence deposited as Patent Deposit No. PTA-75;

i) a sunflower BBE;

j) the amino acid sequence set forth in SEQ ID:2;

k) an amino acid sequence that shares at least 60% sequence similarity to the sequence of SEQ ID:2;

l) an amino acid sequence encoded by the nucleotide sequence deposited as Patent Deposit No. PTA-73.

20. A promoter capable of driving expression in a plant cell said promoter selected from the group consisting of:

a) a promoter that drives expression of a sunflower PR5 gene in its native state;

b) a promoter whose sequence is immediately 5′ to the sequence set forth in SEQ ID:4 in its native state;

c) a promoter having the sequence set forth in SEQ ID:7;

d) a promoter that drives expression of a sunflower defensin gene in its native state;

e) a promoter having the sequence set forth in SEQ ID:9;

f) a promoter whose sequence is immediately 5′ to the sequence set forth in SEQ ID:6 in its native state;

g) a promoter having the nucleotide sequence deposited as Patent Deposit No. PTA-560;

h) a promoter that drives expression of a sunflower BBE;

i) a promoter whose sequence is immediately 5′ to the sequence set forth in SEQ ID:5 in its native state; and

j) a promoter having the sequence set forth in SEQ ID:8.

21. A DNA construct comprising a promoter of claim 20.

22. A vector comprising a nucleotide sequence of claim 20.

23. A plant comprising a nucleotide sequence of claim 20 stably incorporated in its genome.

24. A plant cell comprising a nucleotide sequence of claim 20 stably incorporated in its genome.

25. A composition comprising a protein of claim 19, and a carrier.

26. The composition of claim 25, wherein said carrier is selected from a surface active agent, an inert carrier, an encapsulating agent and an agrochemical pharmaceutical carrier.

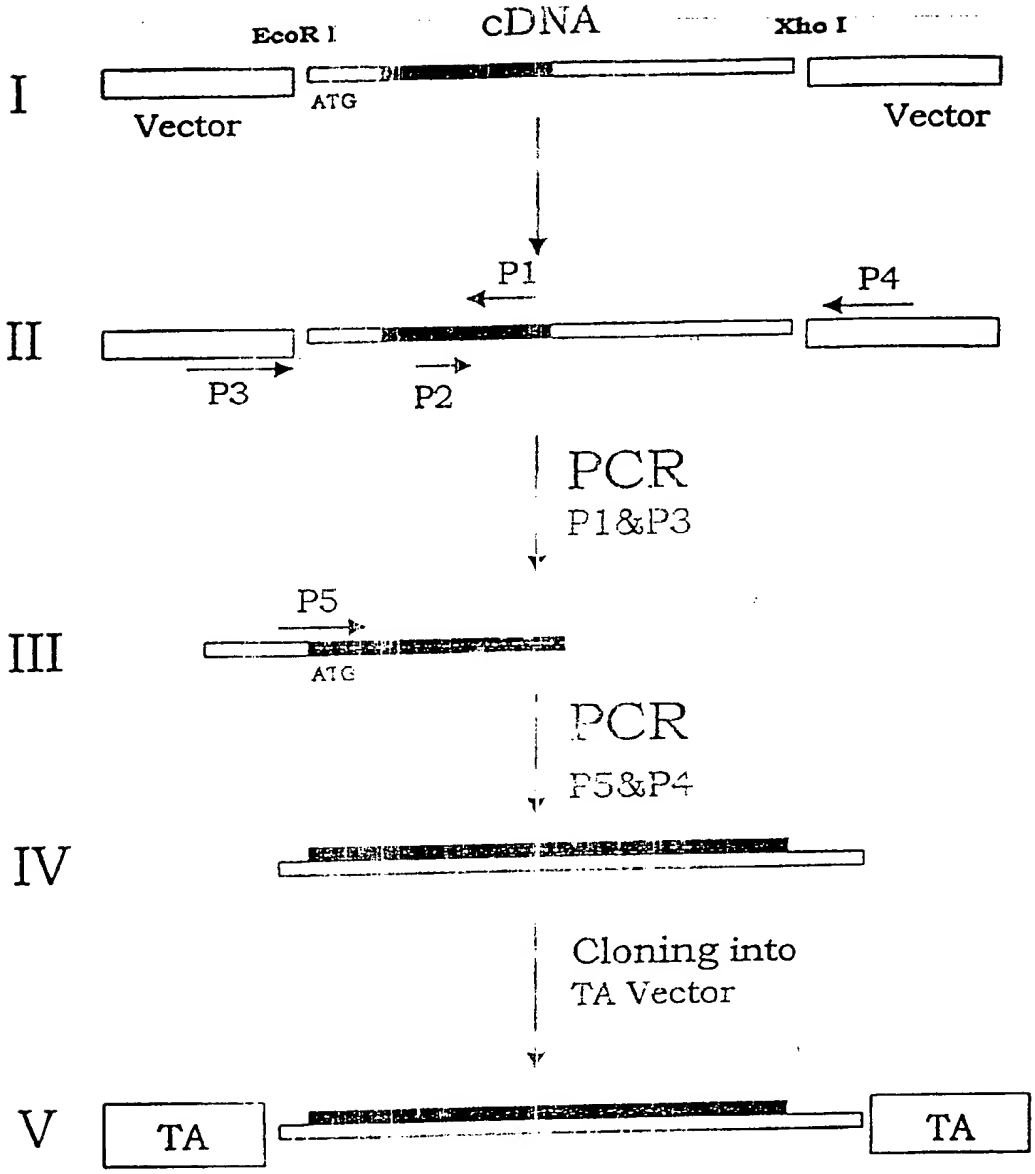
27. The composition of claim 25, wherein said carrier is a pharmaceutical carrier.

28. A method for controlling a plant pathogen comprising applying an anti-pathogenic amount of the protein of claim 19 to the environment of said pathogen.

29. The method of claim 28 wherein said protein is applied to a plant.

30. The method of claim 28 wherein said protein is applied by a procedure selected from the group consisting of spraying, dusting, scattering and seed coating.

31. A method for controlling a plant pathogen comprising applying an anti-pathogenic amount of the composition of claim 25 to the environment of said pathogen.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Vector
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P1
P4
P3
P2
PCR
P1&P3
III
P5
ATG
PCR
P5&P4
IV
Cloning into
TA Vector
V
TA
TA

FIGURE 1

```
CLUSTAL W (1.7) multiple sequence alignment


P93621          MGLCKILSISSFLLTTLFFTSSYAATFNIQNHCSYTVWAAAVP-GGGMQLGSGQSWSLNV
O04708          MRFTTTLPILIPLLLSLLFTSTHAATFDILNKCTYTVWAAASP-GGGRRLDSGQSWTITV
P25096          ------------------------ARFEITNRCTYTVWAASVPVGGGVQLNPGQSWSVDV
PR5-1sun        ---MITSTLPTFLLLAILFHYTNAAVFTIRNNCPYTVWAGAVP-GGGRQLNSGQTWSLTV
Q01591          ----------FFFLLAFVTYTYAATFEVRNNCPYTVWAASTPIGGGRRLDRGQTWVINA
P50701          ---MAYLRSSFVFFLLAFVTYTYAATIEVRNNCPYTVWAASTPIGGGRRLDRGQTWVINA
                                          * : : *.*.*****.: * *** :*. **:* : .

P93621          NAGTTGARVWGRTNCNFDASGNGKCETGDCGGLLQCTAYGTPPNTL-AEFALNQFSNLDF
O04708          NPGTTNARIWGRTSCTFDANGRGKCETGDCNGLLECQGYGSPPNTL-AEFALNQPNNLDY
P25096          PAGTKGARVWARTGCNFDGSGRGGCQTGDCGGVLDCKAYGAPPNTL-AEYGLNGFNNLDF
PR5-1sun        AAGTAGARIWPRTNCNFDGSGRGRCQTGDCNGLLQCQNYGTPPNTFGSEYALNQFNNLDF
Q01591          PRGTKMARIWGRTNCNFDGDGRGSCQTGDCGGVLQCTGWGKPPNTL-AEYALDQFSNLDF
P50701          PRGTKMARIWGRTNCNFDGAGRGSCQTGDCGGVLQCTGWGKPPNTL-AEYALDQFSNLDF
                  **  **:* **.*.**. *.* *:****.*:*:*  :* ****: :*:.*:  .***:

P93621          FDISLVDGFNVPMAFNPT--SNGCTRGISCTADIVGECPAALKTTGGCNNPCTVFKTDEY
O04708          IDISLVDGFNIPMDFS------GC-KGIQCSVDINGQCPSELKAPGGCNNPCTVFKTNEY
P25096          FDISLVDGFNVPMDFSPT--SNGCTRGISCTADINGQCPSELKTQGGCNNPCTVFKTDQY
PR5-1sun        FDISLVDGFNVPMVFRPN--SNGCTRGISCTADINGQCPGELRAPGGCNNPCTVYKTDQY
Q01591          WDISLVDGFNIPMTFAPTNPSGGKCHAIHCTANINGECPGSLRVPGGCNNPCTTFGGQQY
P50701          WDISLVDGFNIPMTFAPTNPSGGKCHAIHCTANINGECPGSLRVPGGCNNPCTTFGGQQY
                 *********:** *       *  :.* *:.:* *:**. *:. ********.:  ::*

P93621          CCNSG--SCNATTYSEFFKTRCPDAYSYPKDDQTSTFTCPAG-TNYEVIFCP---------
O04708          CCTQGGGCGPTTYSKFFKNRCPDAYSYPQDDKTSLFTCPSG-TNYKVTFCP---------
P25096          CCNSG--SCGPTSYSRFFKQRCPDAYSYPKDDPPSTFTCNGG-TDYRVVFCP---------
PR5-1sun        CCNSG--NCGPTDLSRFFKTRCPDAYSYPKDDPTSTFTCPGG-TNYDVIFCP---------
Q01591          CCTQG--PCGPTDLSRFFKQRCPDAYSYPQDDPTSTFTCPSGSTNYRVVFCPNGVTSPNF
P50701          CCTQG--PCGPTDLSRFFKQRCPDAYSYPQDDPTSTFTCPSGSTNYRVVFCPNGVTSPNF
                **..*   *..*  *.*** *********:** .* *** .* *:* * ***

P93621          ----------
O04708          ----------
P25096          ----------
PR5-1sun        ----------
Q01591          PLEMPSSDEEAH
P50701          PLEMPASDEEAH
```

FIGURE 2

CLUSTAL W (1.7) multiple sequence alignment

```
P30986          -MENKTPIFFSLSIFLS------LLNCALGG--ND--LLSCLTFNGVRNHTV---FSADS
P93479          MMCRSLTLRFFLFIVL-------LQTCVRGGDVNDNLLSSCLNSHGVHNFTT---LSTDT
Sunf-19         ---METSILTLLLLLLS------TQSSATSRSITDR-FIQCLHDRADPSFPITGEVYTPG
Sunf-15         ---MQTSILTLLLLLLS------TQSSATSRSITDR-FIQCLHDRADPSFPITGEVYTPG
BBE             --MNNSRSVFLLVLALSFCVSFGALSSIFDVTSTSEDFITCLQSNSNNVTTISQLVFTPA
                     .     * : *          .. .  .. : ** ..    .   . :

P30986          DSDFNRFLHLSIQNPLFQNSLISKPSAIILPGSKEELSNTIRCIRKGSWTIRLRSGGHSY
P93479          NSDYFKLLHASMQNPLFAKPTVSKPSFIVMPGSKEELSSTVHCCTRESWTIRLRSGGHSY
Sunf-19         NSSFPTVLQNYIRNLRFNETTTPKPFLIITAEHVSHIQAAVVCGKQNRLLLKTRSGGHDY
Sunf-15         NSSFPTVLQNYIRNLRFNETTTPKPFLIITAEHVSHIQAAVVCGKQNRLLLKTRSGGHDY
BBE             NTSYIPIWQAANDPIRFNKSTIPKPSVIVTPTDETQIQTALLCAKKHGYEFRIRDGGHDF
                ::.:  . :       * :.  .**  *: .    .:. :: *  :    :: *.***.:

P30986          EGLSYISDI--PFILIDLMNLNRVSIDLESETAWVESGSTLGELYYAITESSSKLGFTAG
P93479          EGLSYTADT--PFVIVDMMNLNRISIDVLSETAWVESGATLGELYYAIAQSTDTLGFTAG
Sunf-19         EGLSYLTNTNQPFFIVDMFNLRSINVDIEQETAWVQAGATLGEVYYRIAEKSNKHGFPAG
Sunf-15         EGLSYLTNTNQPFFIVDMFNLRSINIDIEQETAWVQAGATLGEVYYRIAEKSNKHGFPAG
BBE             EGNSYFANA--PFVMLDLVNMRAIEINVENRTALVQGGALLGELYYTISQKTDTLYFPAG
                ** ** :::  **.::*:.*:. :.::: ..** *:.*: ***:** *::.:..  *.**

P30986          WCPTVGIGGHISGGGFGMMSRKYGLAADNVVDAILIDANGAILDRQAMGEDVFWAIRGGG
P93479          WCPIVGSGGHISGGGFGMMSRKYGLAADNVVDAILIDSNGAILDREKMGDDVFWAIRGGG
Sunf-19         VCPIVGVGGHFSGGGYGNLMRKYGLSVDNIVDAQIIDVNGKLLDRKSMGEDLFWAYTGGG
Sunf-15         VCPIVGVGGHFSGGGYGNLMRKYGLSVDNIVDAQIIDVNGKLLDRKSMGEDLFWAITGGG
BBE             IWAGVGVVSGFLSGGGYGNLLRKYGLGADNVLDIRIMDVNGNILDRKSMGEDLFWALRGGG
                  . ** .*.:****:* : *****..**::*  ::* ** :***: **:*:***  ***

P30986          GGVWGAIYAWKIKLLPVPEKVTVFRVTKNVAIDEATSLLHKWQFVAEELEED------FT
P93479          GGVWGAIYAWKIKLLPVPEKLTVFRVTKNVGIEDASSLLHKWQYVADELDED------FT
Sunf-19         GVSFGVIIAYKIKLVRVPEVVTVFTIER-REEQNLSTIAERWVQVADKLDRDLFL--RMT
Sunf-15         GVSFGVIIAYKIKLVRVPEVVTVFTIER-REEQNLSTIAERWVQVADKLDRDLFL--RMT
BBE             ASSFGIVLQWKLNLVPVPERVTLFSVSY-TLEQGATDIFHKYQYVLPKFDRDLLIRVQLN
                .  :* :  :*::*: *** :*:* :      :  : : .::  *  :::.*      :.

P30986          LSVLGGRDE-KQVWLTMLGFHFGLNTVAKSTFDLLFPELGLVEEDYLEMSWGESFAYLAG
P93479          VSVLGGVNG-NDAWLMFLGLHLGRKDAAKTIIDEKFPELGLVDKEFQEMSWGESMAFLSG
Sunf-19         FSVIHDTNGGETVRAIFPILYLGNSRMLVTLLNKDFPELGLQESDCTEMSWVESVLYYTG
Sunf-15         FSVIHDTNGGETVRAIFPILYLGNSRMLVTLLNKDFPELGLQESDCTEMSWVESVLYYTG
BBE             TEYIGNTTQ-KTVRILFHGIYQGNIDTLLPLLNQSFPELNVTREVCQEVRMVQTTLEFGG
                 . :...   : .  :  :: *      . ::  ****.:  .   *:   ::     *

P30986          LETYSQINNRFLKFDERA--FKTKVDLTKEPLPSKAFYGLLERLSKEPN-GFIALNGFGG
P93479          LDTLSQINNRFLKFDERA--FKTKVDFTKVSVPLNVFFHALEMLSEQPG-GFIALNGFGG
Sunf-19         FPSGITTTALLSRTPQRLNPFKIKSDYVQNPISKRQFEFIFERMKELEN-QMLAFNPYGG
Sunf-15         FPSGITTTALLSRTPQRLNPFFIKSDYVQNPISKRQFEFIFERLKELEN-QMLAFNPYGG
BBE             FNISTPFSVLANRSAIPKLSFKGKSDYVRIPIPRSGLRKLWRKMFENDNSQTLFMYTFGG
                :   :  .   :       ** * * .: .::.  :    . : :  .   : :  :**
```

FIGURE 3 (1)

```
P30986          QMSKISSDFTPFPHRSGTRLMVEYIVAWNQSEQKK---KTEFLDWLEKVYEFMKPFVSKN
P93479          KMSEISTDFTPFPHRKGTKLMFEYIIAWNQDEESK---IGEFSEWLAKFYDYLEPFVSKE
Sunf-19         RMSEISTDFAPFPHRSGNIAKIQYEVNWEDLSDEA---ENRYLNFTRLMYDYMTPFVSKN
Sunf-15         RMSEISTDFAPFPHRSGNIAKIQYEVNWEDLSDEA---ENRYLNFTRLMYDYMTPFVSKN
BBE             KMEEYSDTAIPYPHRAGVLYQVFKPVTFVDQPSDKTLISLRRLAWLRSFDKTLEPYVTSN
                :*.: *    :.*** *    .  : : :  ..      .  :   . . : *:*:.:

P30986          PRLGYVNHIDLDLGGIDWGNKTVVNNAIEISRSWGESYF-LSNYERLIRAKTLIDPNNVF
P93479          PRVGYVNHIDLDIGGIDWRNKSSTTNAVEIARNWGERYF-SSNYERLVKAKTLIDPNNVF
Sunf-19         PREAFLNYRDLDIG-INSHGR----NAYTEGMVYGHKYFKETNYKRLVSVKTKVDPDNFF
Sunf-15         PRKAFLNYRDLDIG-INSHGR----NAYTEGMVYGHKYFKETNYKRLVSVKTKVDPDNFF
BBE             PREAYMNYNDLDLG--FDS------AAYEEASEWGERYWKRENFKKLIRIKAKVDPENFF
                *: .:*:. .*:* *             *   .  :*. *:   *:::*:  *: :**:*.*

P30986          NHPQSIPPMANFD--YLEKTLGSDGGEVVI
P93479          NHPQSIPPMMKFEEIYMLKEL---------
Sunf-19         RNEQSIPPLLS----------------------
Sunf-15         RNEQSIPPLLS----------------------
BBE             RHPQSIPVFSPPLSDM-------------
                .: **** :
```

FIGURE 3 (2)

```
CLUSTAL W (1.7) multiple sequence alignment

P30230          MAKFASIIVLLFVALVVFAAFEEPTMVEAQKLCQRPSGTWSGVCGNNNACKNQCIRLEKA
P30231          ------------------------------QKLCERPSGTWSGVCGNNNACKNQCINLEKA
P30224          MAKSATIVTLFFAALVFFAALEAPMVVEAQKLCERPSGTWSGVCGNSNACKNQCINLEKA
defensin        MAKISVAFNAFLLLLFVLAISEIGSVKG--ELCEKASQTWSGTCGKTKHCDDQCKSWEGA
Q01784          MEKKSLAALSFLLLLVLFVAQEIVVTEA--NTCEHLADTYRGVCFTNASCDDHCKNKAHL
                                              : *:: : *: *.* ..  *.::*

P30230          RHGSCNYVFPAHKCICYFPC--------------------------------
P30231          RHGSCNYVFPAHKCICYFPC--------------------------------
P30224          RHGSCNYVFPAHKCICYFPC--------------------------------
defensin        AHGACHVRDGKHMCFCYFNCSKAQKLAQDKLRAEELAKEKIEPEKATAKP
Q01784          ISGTCHD----WKCFCTQNC--------------------------------
                  *:*:       *:*   *
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FIGURE 4

SEQUENCE LISTING

<110> Bidney, Dennis L.
Crasta, Oswald R.
Duvick, Jon
Hu, Xu
Lu, Guihua

<120> Sunflower Anti-Pathogenic Proteins and Genes and their Uses

<130> 5718-90-1

<150> 60/140,646
<151> 1999-06-23

<150> 60/162,904
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            20                  25                  30
Thr Val Trp Ala Gly Ala Val Pro Gly Gly Gly Arg Gln Leu Asn Ser
        35                  40                  45
Gly Gln Thr Trp Ser Leu Thr Val Ala Ala Gly Thr Ala Gly Ala Arg
    50                  55                  60
Ile Trp Pro Arg Thr Asn Cys Asn Phe Asp Gly Ser Gly Arg Gly Arg
65                  70                  75                  80
Cys Gln Thr Gly Asp Cys Asn Gly Leu Leu Gln Cys Gln Asn Tyr Gly
                85                  90                  95
Thr Pro Pro Asn Thr Leu Ala Glu Tyr Ala Leu Asn Gln Phe Asn Asn
            100                 105                 110
Leu Asp Phe Phe Asp Ile Ser Leu Val Asp Gly Phe Asn Val Pro Met
        115                 120                 125
Val Phe Arg Pro Asn Ser Asn Gly Cys Thr Arg Gly Ile Ser Cys Thr
    130                 135                 140
Ala Asp Ile Asn Gly Gln Cys Pro Gly Glu Leu Arg Ala Pro Gly Gly
145                 150                 155                 160
Cys Asn Asn Pro Cys Thr Val Tyr Lys Thr Asp Gln Tyr Cys Cys Asn
                163                 170                 175
Ser Gly Asn Cys Gly Pro Thr Asp Leu Ser Arg Phe Phe Lys Thr Arg
            180                 185                 190
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            20                  25                  30
Ser Glu Asp Phe Ile Thr Cys Leu Gln Ser Asn Ser Asn Asn Val Thr
        35                  40                  45
Thr Ile Ser Gln Leu Val Phe Thr Pro Ala Asn Thr Ser Tyr Ile Pro
    50                  55                  60
Ile Trp Gln Ala Ala Ala Asp Pro Ile Arg Phe Asn Lys Ser Tyr Ile
65                  70                  75                  80
Pro Lys Pro Ser Val Ile Val Thr Pro Thr Asp Glu Thr Gln Ile Gln
                85                  90                  95
Thr Ala Leu Leu Cys Ala Lys Lys His Gly Tyr Glu Phe Arg Ile Arg
            100                 105                 110
Asp Gly Gly His Asp Phe Glu Gly Asn Ser Tyr Thr Ala Asn Ala Pro
        115                 120                 125
Phe Val Met Leu Asp Leu Val Asn Met Arg Ala Ile Glu Ile Asn Val
    130                 135                 140
Glu Asn Arg Thr Ala Leu Val Gln Gly Gly Ala Leu Leu Gly Glu Leu
145                 150                 155                 160
Tyr Tyr Thr Ile Ser Gln Lys Thr Asp Thr Leu Tyr Phe Pro Ala Gly
                165                 170                 175
Ile Trp Ala Gly Val Gly Val Ser Gly Phe Leu Ser Gly Gly Gly Tyr
            180                 185                 190
Gly Asn Leu Leu Arg Lys Tyr Gly Leu Gly Ala Asp Asn Val Leu Asp
        195                 200                 205
Ile Arg Phe Met Asp Val Asn Gly Asn Ile Leu Asp Arg Lys Ser Met
    210                 215                 220
Gly Glu Asp Leu Phe Trp Ala Leu Arg Gly Gly Gly Ala Ser Ser Phe
225                 230                 235                 240
Gly Ile Val Leu Gln Trp Lys Leu Asn Leu Val Pro Val Pro Glu Arg
                245                 250                 255
Val Thr Leu Phe Ser Val Ser Tyr Thr Leu Glu Gln Gly Ala Thr Asp
            260                 265                 270
Ile Phe His Lys Tyr Gln Tyr Val Leu Pro Lys Phe Asp Arg Asp Leu
        275                 280                 285
Leu Ile Arg Val Gln Leu Asn Thr Glu Tyr Ile Gly Asn Thr Thr Gln
    290                 295                 300
Lys Thr Val Arg Ile Leu Phe His Gly Ile Tyr Gln Gly Asn Ile Asp
305                 310                 315                 320
Thr Leu Leu Pro Leu Leu Asn Gln Ser Phe Pro Glu Leu Asn Val Thr
                325                 330                 335
Arg Glu Val Cys Gln Glu Val Arg Met Val Gln Thr Thr Leu Glu Phe
            340                 345                 350
Gly Gly Phe Asn Ile Ser Thr Pro Thr Ser Val Leu Ala Asn Arg Ser
        355                 360                 365
Ala Ile Pro Lys Leu Ser Phe Lys Gly Lys Ser Asp Tyr Val Arg Thr
    370                 375                 380
Pro Ile Pro Arg Ser Gly Leu Arg Lys Leu Trp Arg Lys Met Phe Glu
385                 390                 395                 400
Asn Asp Asn Ser Gln Thr Leu Phe Met Tyr Thr Phe Gly Gly Lys Met
                405                 410                 415
Glu Glu Tyr Ser Asp Thr Ala Ile Pro Tyr Pro His Arg Ala Gly Val
            420                 425                 430
Leu Tyr Gln Val Phe Lys Arg Val Asp Phe Val Asp Gln Pro Ser Asp
        435                 440                 445
Lys Thr L u Ile Ser Leu Arg Arg Leu Ala Trp Leu Arg Ser Phe Asp
```

```
    450                 455                 460
Lys Thr Leu Glu Pro Tyr Val Thr Ser Asn Pro Arg Glu Ala Tyr Met
465                 470                 475                 480
Asn Tyr Asn Asp Leu Asp Leu Gly Phe Asp Ser Ala Ala Tyr Glu Glu
                485                 490                 495
Ala Ser Glu Trp Gly Glu Arg Tyr Trp Lys Arg Glu Asn Phe Lys Lys
            500                 505                 510
Leu Ile Arg Ile Lys Ala Lys Val Asp Pro Glu Asn Phe Phe Arg His
        515                 520                 525
Pro Gln Ser Ile Pro Val Phe Ser Arg Pro Leu Ser Asp Met
    530                 535                 540
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<210> 3
<211> 108
<212> PRT
<213> Helianthus annuus

<400> 3

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Met Ala Lys Ile Ser Val Ala Phe Asn Ala Phe Leu Leu Leu Leu Phe
 1               5                  10                  15
Val Leu Ala Ile Ser Glu Ile Gly Ser Val Lys Gly Glu Leu Cys Glu
            20                  25                  30
Lys Ala Ser Gln Thr Trp Ser Gly Thr Cys Gly Lys Thr Lys His Cys
        35                  40                  45
Asp Asp Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
    50                  55                  60
Val Arg Asp Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Ser Lys
65                  70                  75                  80
Ala Gln Lys Leu Ala Gln Asp Lys Leu Arg Ala Glu Glu Leu Ala Lys
                85                  90                  95
Glu Lys Ile Glu Pro Glu Lys Ala Thr Ala Lys Pro
            100                 105
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<210> 4
<211> 875
<212> DNA
<213> Helianthus annuus

<400> 4

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atgacaacct ccaccattcc cactttcctt ctcttggcta ttcttttttca ctataccaat     60
gcagccgtgt tcactattcg aaacaactgt ccatacaccg tttgggctgg tgcggtgcct     120
ggtggcggcc gacaacttaa ctcaggccaa gcctggtctt taaccgtcgc agctggcaca     180
gcaggagccc gtatatggcc ccgaaccaat tgcaactttg atggttctgg gcgaggcagg     240
tgtcagaccg gtgattgcaa cggtctcctc caatgccaaa actatggtac cccacccaac     300
acattggccg agtacgcttt gaaccagttc aacaatcttg atttctttga catttctctt     360
gtggacggat tcaatgtgcc gatggtgttt agacccaatt ctaatgggtg cacccggggt     420
atctcatgta ctgcggatat caatggccag tgtcctggtg agttacgggc tcctggcggg     480
tgcaataacc cttgcaccgt gtacaaaact gatcagtatt gttgcaactc tggaaattgt     540
ggaccaactg atttatcaag gttttcaag accagatgtc ctgatgcata tagttatccc     600
aaggatgatc caactagcac atttacgtgc cccggtggaa ccaactacga cgttatattc     660
tgcccttgat caaagccatt tgattatatg atcaaattaa aaggagttcg aaatataaga     720
actgaaataa atggagtgaa taagtaatgg agatagtcta attataaggc ttcttcctca     780
ttgtaataca atgatgttgt aatttgtcaa aatgaatgga tggatatata tgattaatta     840
ttaggaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa                                875
```

<210> 5
<211> 1809
<212> DNA
<213> Helianthus annuus

<400> 5

aaaacatgaa taactctcgt tcagtgttcc tcttagttct cgctctttca ttttgtgttt 60
catttggagc attgtcttcc atttttgatg ttacttcaac ttccgaagat ttcataacct 120
gtctccaatc caattcaaac aatgtcacca ccatcttcca actcgttttc accccggcca 180
acacttctta catacccatt tggcaagctg cagccgaccc tattcggttc aacaaatcct 240
acattccgaa accatcagtc atcgttactc ccaccgatga aacacagatc caaaccgctc 300
ttttatgcgc caagaaacac ggatatgagt ttaggatccg agacggtggt catgacttcg 360
agggcaactc atacaccgcg aacgctccgt trgtcatgct tgatctcgtc aacatgaggg 420
ctatagagat caacgttgaa aaccggaccg cgctggtcca gggtggcgct ttgcttggtg 480
agctctacta cactatttct cagaaaacgg acaccttgta ttttcctgct ggtatttggg 540
ccggtgtggg tgttagcggg tttttgagcg gtggtgggta tggaaacctg ttgaggaaat 600
acgggcttgg tgccgataat gtcttggata ttcgtttcat ggatgttaat ggaaacattc 660
ttgataggaa atcgatgggc gaagatttgt tttgggcgct tcgtggcggt ggtgcttcca 720
gtttcggaat tgttctccag tggaagctca atttggttcc ggtgcctgaa agagttactc 780
ttttcagtgt gagttatact cnggagcaag gggcgacgga cattttccat aaatatcaat 840
acgtgttacc gaaattttgat cgtgatttac tcatcagagt tcagcttaac accgagtata 900
taggcaacac cactcagaaa accgtacgaa tattgtttca cggtatttat caaggcaata 960
ttgacacact gcttccgttg ttgaaccaaa gtttcccaga ggtcaatgtg acacgagaag 1020
tctgccaaga agtacgaatg gtccagacta ccgttgagtt tggaggcttt aacatctcta 1080
ccccgacatc ggttctagcg aaccgatcag caatccccaa ggtgagcttc aaaggaaaat 1140
ctgactatgt ccgaactcca attcccagaa gcgggctaag aaagctctgg agaaagatgt 1200
ttgaaaacga caactcacag actctcttca tgtcacacatt tggtgggaag atggaggagt 1260
actcagatac agcaattccg tatccccata gagctggggt gttgtaccaa gtgttcaaga 1320
gggtggactt cgtggatcag ccttcggaca agaccttgat atcactcaga cggttagctt 1380
ggctccgaag ctttgataag actttggagc cgtacgtgac gagtaacccg agggaggcgt 1440
atatgaacta caatgatctt gattttgggtt ttgatagtgc tgcatatgaa gaagcaagtg 1500
aatggggaga aaggtattgg aaaagggaga actttaagaa gttgatccga atcaaggcta 1560
aagttgatcc ggaaaatttc tttagacacc cacaaagtat accggttttc tcaagacctc 1620
tctcagatat gtgaagccaa cnctttggat ggtgttcttt ttcttgagta tattggtaat 1680
aattattaat taagaghaaa aagtcgatta ctttttgtgtt tggtgccttg tgtaccaatt 1740
atttaaactt ttttgttttg ataaactttt aatcaaagct attatgtatt taaaaaaaaa 1800
aaaaaaaaa 1809

<210> 6
<211> 565
<212> DNA
<213> Helianthus annuus

<400> 6
tcggcttgag cttgagctta gttcagtaac ttaaaaatgg ccaaaatttc agttgctttc 60
aatgcttttc ttctgcttct ctttgttctt gctatctcag aaatcggatc ggtgaaagga 120
gaattatgtg agaaggcaag cnagacatgg tccggaacat gtggcaagac aaaacactgt 180
gatgaccagt gcaagtcttg ggagggtgca gcccanggag cttgtcacgt gcgcgatggg 240
aaacacatgt gcttctgcta cttcaactgt tccaaagccc agaagttggc tcaggataaa 300
ctcagagcgg aagagctcgc caaggagaag attgaacccg aaaaggcgac agccaaacct 360
tgagtatgta gcaaatgtca tacgattatg aataaagaga aaatgctttc tacttggcat 420
attcagcatt ttcttgtgtg taatgtttgt tgtatttgga aattggaatc agttgcttca 480
ttatgattcc atgcaaaatg ttctaatgaa atgatattta aattaaaaaa aaaaaaaaaa 540
aaaaaaaaaa aaaaaaaaaa aaaaa 565

<210> 7
<211> 550
<212> DNA
<213> Helianthus annuus

<400> 7
aaaactgcgt tttgtaaagg catgtaggta catgcttttc cgaaactgcg ttttgaaggc 60
agataatcac ttttgatcca aacattttttt tagattactt atgttttaca aacgcaatac 120
ttaaataacc acttaaaaac gtaatcccaa acacctctct agtgtataaa aaacctgaaa 180
ttagtttata cacaaagaaa ataacaaatt aaaagcataa acaaaaatga taattttata 240
aatgataaac aaaagcaagt ataagaataa gataatatat attttttata gagttactaa 300
atacaaagat aaaatcacaa aaaagagtaa actaaaataa gttataacaa atgtgttgtt 360

aactgtatag ttatgatttt gtctactaca gaacaattcc acgtaaccat tttgttcaat 420
gaatacattt gaaatttcaa tgaatgtata tctttctaaa tattgtacgt atagcatgtt 480
cggcctatat aaaccatgtt tactctcact tccaattcac ccaaaaccac aatgacaacc 540
tccacccttc 550

<210> 8
<211> 351
<212> DNA
<213> Helianthus annuus

<400> 8
tgttcaaaaa agcacggtga aagtgactaa tattcgaatc tagtcgtgac cgctgcctag 60
tgccaaatta ctaaaggaga agaaaaaaaa aatatcaaga agatacagaa aattatttgc 120
gcgttgtgac ttgtgttgtg tagtcaacgg gcatctagtc atacatttga tggctgtttc 180
ggtgtaaaca taagtcaaag gctagatgtc tttttactaa aaaggttgtt ttagtaattt 240
cccaaaaaaa catccaactt tcttccttat tttttccaaa tcgccttcgg gttcatctta 300
tataaatagg cgcattaagt gctaatagac tcaccaaacc aacaaaacat g 351

<210> 9
<211> 1346
<212> DNA
<213> Helianthus annuus

<400> 9
gtacttcgca aaagggcctg tcgtaaatgt tagtgagatg ggcaatcgtc tcaacgtcaa 60
tatttagcga ccataatttg tcagaaaatt aacgacagga actaaattga ttgcaaattt 120
agcaaccaaa ataatcattc tgaaagtaat tagcaattaa acaaaattgg tggtaaattt 180
acaacgattt ttttttcaat gatttattgt tattttttac ttgacctgac tactgagttg 240
ttttaacctt aatcattttt atcagagtga ataaagcctc catggcacag aaaaaatgta 300
agaattatat gaataaagaa aattacgata atttttatta taaataggtg gtttaggaaa 360
actattaagc cctgttgttt tgcatctgaa tagaatcaat cagaggttgg ctctgattca 420
atcagaactc aaaagtttcg gtgtttggtt cgacatctca atgacatcta aatggggatt 480
tcaagctctt aacaattcag ctttgaggag tcgctaaacc atttagagga tttctgatat 540
tacatgtaaa aattaattaa agtcacgtgc atgtgtatat gaatgaattt catcaaagtc 600
gcgtgcaagt gtatatgtta tctgaatggt cctgtatcta atatacaaac atatgtttac 660
atgcaatttc aaaaatgccc taacccacgt agtgacacaa aaaaaaaaaa aaaagtttgc 720
taacttatga aagttacttg gatgtataca atgcacgcac cacaaaagtc aatttaagac 780
aaattttgtg gaaactttag ccattttgtg tttattgttt attgtttatt ttcttgactt 840
tcaacatatt ttctcctata aatacccctc attgtctcat cttctcttca caaaccttgc 900
aacaagtgtt cttcagctta gttcagtaac taaaaaatgg ccaaaagtgc agttgctttc 960
tatgcttttc ttcccatttc ctttgttctt gctatctcag gttctcaatc aatcttattt 1020
acactcactt tgtgtttgta atattcagac tcttacatct taatgtcaca tattttgacc 1080
cttcggatga caattagttt agttaagtag accgtgacat taagctagca ctcatactta 1140
aataatgcag tgaaaagaag cattttataa gtatataaaa gtgatttaat tagcttttat 1200
ttcgtgcaga aactaatcat attcatcaca aaactgcatt cgttagacat tctagatttg 1260
tgtataacgt acttaacaca gtttaacgtg tacagaaatc ggatcggtga aaggagaatt 1320
atgtgagaag gcaagccaga catgtc 1346

<210> 10
<211> 225
<212> PRT
<213> Vitis vinifera

<400> 10
Met Gly Leu Cys Lys Ile Leu Ser Ile Ser Ser Phe Leu Leu Thr Thr
1 5 10 15
Leu Phe Phe Thr Ser Ser Tyr Ala Ala Thr Phe Asn Ile Gln Asn His
20 25 30
Cys Ser Tyr Thr Val Trp Ala Ala Ala Val Pro Gly Gly Gly Met Gln
35 40 45
Leu Gly Ser Gly Gln Ser Trp Ser Leu Asn Val Asn Ala Gly Thr Thr

50 55 60
Gly Ala Arg Val Trp Gly Arg Thr Asn Cys Asn Phe Asp Ala Ser Gly
65 70 75 80
Asn Gly Lys Cys Glu Thr Gly Asp Cys Gly Gly Leu Leu Gln Cys Thr
85 90 95
Ala Tyr Gly Thr Pro Pro Asn Thr Leu Ala Glu Phe Ala Leu Asn Gln
100 105 110
Phe Ser Asn Leu Asp Phe Phe Asp Ile Ser Leu Val Asp Gly Phe Asn
115 120 125
Val Pro Met Ala Phe Asn Pro Thr Ser Asn Gly Cys Thr Arg Gly Ile
130 135 140
Ser Cys Thr Ala Asn Ile Val Gly Glu Cys Pro Ala Ala Leu Lys Thr
145 150 155 160
Thr Gly Gly Cys Asn Asn Pro Cys Thr Val Phe Lys Thr Asp Glu Tyr
165 170 175
Cys Cys Asn Ser Gly Ser Cys Asn Ala Thr Thr Tyr Ser Glu Phe Phe
180 185 190
Lys Thr Arg Cys Pro Asp Ala Tyr Ser Tyr Pro Lys Asp Asp Gln Thr
195 200 205
Ser Thr Phe Thr Cys Pro Ala Gly Thr Asn Tyr Glu Val Ile Phe Cys
210 215 220
Pro
225

<210> 11
<211> 224
<212> PRT
<213> Vicia villosa

<400> 11
Met Arg Phe Thr Thr Thr Leu Pro Ile Leu Ile Pro Leu Leu Leu Ser
1 5 10 15
Leu Leu Phe Thr Ser Thr His Ala Ala Thr Phe Asp Ile Leu Asn Lys
20 25 30
Cys Thr Tyr Thr Val Trp Ala Ala Ala Ser Pro Gly Gly Gly Arg Arg
35 40 45
Leu Asp Ser Gly Gln Ser Trp Thr Ile Thr Val Asn Pro Gly Thr Thr
50 55 60
Asn Ala Arg Ile Trp Gly Arg Thr Ser Cys Thr Phe Asp Ala Asn Gly
65 70 75 80
Arg Gly Lys Cys Glu Thr Gly Asp Cys Asn Gly Leu Leu Glu Cys Gln
85 90 95
Gly Tyr Gly Ser Pro Pro Asn Thr Leu Ala Glu Phe Ala Leu Asn Gln
100 105 110
Pro Asn Asn Leu Asp Tyr Ile Asp Ile Ser Leu Val Asp Gly Phe Asn
115 120 125
Ile Pro Met Asp Phe Ser Gly Cys Arg Gly Ile Gln Cys Ser Val Asp
130 135 140
Ile Asn Gly Gln Cys Pro Ser Glu Leu Lys Ala Pro Gly Gly Cys Asn
145 150 155 160
Asn Pro Cys Thr Val Phe Lys Thr Asn Glu Tyr Cys Cys Thr Asp Gly
165 170 175
Pro Gly Ser Cys Gly Pro Thr Thr Tyr Ser Lys Phe Phe Lys Asp Arg
180 185 190
Cys Pro Asp Ala Tyr Ser Tyr Pro Gln Asp Asp Lys Thr Ser Leu Phe
195 200 205
Thr Cys Pro Ser Gly Thr Asn Tyr Lys Val Thr Phe Cys Pro
210 215 220

<210> 12
<211> 202

<212> PRT
<213> Glycine Max

<400> 12

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Ala Arg Phe Glu Ile Thr Asn Arg Cys Thr Tyr Thr Val Trp Ala Ala
 1               5                  10                  15
Ser Val Pro Val Gly Gly Gly Val Gln Leu Asn Pro Gly Gln Ser Trp
            20                  25                  30
Ser Val Asp Val Pro Ala Gly Thr Lys Gly Ala Arg Val Trp Ala Arg
        35                  40                  45
Thr Gly Cys Asn Phe Asp Gly Ser Gly Arg Gly Gly Cys Gln Thr Gly
    50                  55                  60
Asp Cys Gly Gly Val Leu Asp Cys Lys Ala Tyr Gly Ala Pro Pro Asn
65                  70                  75                  80
Thr Leu Ala Glu Tyr Gly Leu Asn Gly Phe Asn Asn Leu Asp Phe Phe
                85                  90                  95
Asp Ile Ser Leu Val Asp Gly Phe Asn Val Pro Met Asp Phe Ser Pro
            100                 105                 110
Thr Ser Asn Gly Cys Thr Arg Gly Ile Ser Cys Thr Ala Asp Ile Asn
        115                 120                 125
Gly Gln Cys Pro Ser Glu Leu Lys Thr Gln Gly Gly Cys Asn Asn Pro
    130                 135                 140
Cys Thr Val Phe Lys Thr Asp Gln Tyr Cys Cys Asn Ser Gly Ser Cys
145                 150                 155                 160
Gly Pro Thr Asp Tyr Ser Arg Phe Phe Lys Gln Arg Cys Pro Asp Ala
                165                 170                 175
Tyr Ser Tyr Pro Lys Asp Asp Pro Pro Ser Thr Phe Thr Cys Asn Gly
            180                 185                 190
Gly Thr Asp Tyr Arg Val Val Phe Cys Pro
        195                 200
```

<210> 13
<211> 223
<212> PRT
<213> Helainthus annuus

<400> 13

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Met Thr Thr Ser Thr Leu Pro Thr Phe Leu Leu Leu Ala Ile Leu Phe
 1               5                  10                  15
His Tyr Thr Asn Ala Ala Val Phe Thr Ile Arg Asn Asn Cys Pro Tyr
            20                  25                  30
Thr Val Trp Ala Gly Ala Val Pro Gly Gly Gly Arg Gln Leu Asn Ser
        35                  40                  45
Gly Gln Thr Trp Ser Leu Thr Val Ala Ala Gly Thr Ala Gly Ala Arg
    50                  55                  60
Ile Trp Pro Arg Thr Asn Cys Asn Phe Asp Gly Ser Gly Arg Gly Arg
65                  70                  75                  80
Cys Gln Thr Gly Asp Cys Asn Gly Leu Leu Gln Cys Gln Asn Tyr Gly
                85                  90                  95
Thr Pro Pro Asn Thr Phe Gly Ser Glu Tyr Ala Leu Asn Gln Phe Asn
            100                 105                 110
Asn Leu Asp Phe Phe Asn Ile Ser Leu Val Asp Gly Phe Asn Val Pro
        115                 120                 125
Met Val Phe Arg Pro Asn Ser Asn Gly Cys Thr Arg Gly Ile Ser Cys
    130                 135                 140
Thr Ala Asp Ile Asn Gly Gln Cys Pro Gly Glu Leu Arg Ala Pro Gly
145                 150                 155                 160
Gly Cys Asn Asn Pro Cys Thr Val Tyr Lys Thr Asp Gln Tyr Cys Cys
                165                 170                 175
Asn Ser Gly Asn Cys Gly Pro Thr Asp Leu Ser Arg Phe Phe Lys Thr
            180                 185                 190
```

Arg Cys Pro Asp Ala Tyr Ser Tyr Pro Lys Asp Asp Pro Thr Ser Thr
195 200 205
Phe Thr Cys Pro Gly Gly Thr Asn Tyr Asp Val Ile Phe Cys Pro
210 215 220

<210> 14
<211> 238
<212> PRT
<213> Lycopersicon esculentum

<400> 14
Phe Phe Phe Leu Leu Ala Phe Val Thr Tyr Thr Tyr Ala Ala Thr Phe
1 5 10 15
Glu Val Arg Asn Asn Cys Pro Tyr Thr Val Trp Ala Ala Ser Thr Pro
20 25 30
Ile Gly Gly Gly Arg Arg Leu Asp Arg Gly Gln Thr Trp Val Ile Asn
35 40 45
Ala Pro Arg Gly Thr Lys Met Ala Arg Ile Trp Gly Arg Thr Asn Cys
50 55 60
Asn Phe Asp Gly Asp Gly Arg Gly Ser Cys Gln Thr Gly Asp Cys Gly
65 70 75 80
Gly Val Leu Gln Cys Thr Gly Trp Gly Lys Pro Pro Asn Thr Leu Ala
85 90 95
Glu Tyr Ala Leu Asp Gln Phe Ser Asn Leu Asp Phe Trp Asp Ile Ser
100 105 110
Leu Val Asp Gly Phe Asn Ile Pro Met Thr Phe Ala Pro Thr Asn Pro
115 120 125
Ser Gly Gly Lys Cys His Ala Ile His Cys Thr Ala Asn Ile Asn Gly
130 135 140
Glu Cys Pro Gly Ser Leu Arg Val Pro Gly Gly Cys Asn Asn Pro Cys
145 150 155 160
Thr Thr Phe Gly Gly Gln Gln Tyr Cys Cys Thr Gln Gly Pro Cys Gly
165 170 175
Pro Thr Asp Leu Ser Arg Phe Phe Lys Gln Arg Cys Pro Asp Ala Tyr
180 185 190
Ser Tyr Pro Gln Asp Asp Pro Thr Ser Thr Phe Thr Cys Pro Ser Gly
195 200 205
Ser Thr Asn Tyr Arg Val Val Phe Cys Pro Asn Gly Val Thr Ser Pro
210 215 220
Asn Phe Pro Leu Glu Met Pro Ser Ser Asp Glu Glu Ala Lys
225 230 235

<210> 15
<211> 246
<212> PRT
<213> Solanum commersonii

<400> 15
Met Ala Tyr Leu Arg Ser Ser Phe Val Phe Phe Leu Leu Ala Phe Val
1 5 10 15
Thr Tyr Thr Tyr Ala Ala Thr Ile Glu Val Arg Asn Asn Cys Pro Tyr
20 25 30
Thr Val Trp Ala Ala Ser Thr Pro Ile Gly Gly Gly Arg Arg Leu Asp
35 40 45
Arg Gly Gln Thr Trp Val Ile Asn Ala Pro Arg Gly Thr Lys Met Ala
50 55 60
Arg Ile Trp Gly Arg Thr Asn Cys Asn Phe Asp Gly Ala Gly Arg Gly
65 70 75 80
Ser Cys Gln Thr Gly Asp Cys Gly Gly Val Leu Gln Cys Thr Gly Trp
85 90 95
Gly Lys Pro Pro Asn Thr Leu Ala Glu Tyr Ala Leu Asp Gln Phe Ser

```
                100                 105                 110
Asn Leu Asp Phe Trp Asp Ile Ser Leu Val Asp Gly Phe Asn Ile Pro
        115                 120                 125
Met Thr Phe Ala Pro Thr Asn Pro Ser Gly Gly Lys Cys His Ala Ile
    130                 135                 140
His Cys Thr Ala Asn Ile Asn Gly Glu Cys Pro Gly Ser Leu Arg Val
145                 150                 155                 160
Pro Gly Gly Cys Asn Asn Pro Cys Thr Thr Phe Gly Gly Gln Gln Tyr
                165                 170                 175
Cys Cys Thr Gln Gly Pro Cys Gly Pro Thr Asp Leu Ser Arg Phe Phe
            180                 185                 190
Lys Gln Arg Cys Pro Asp Ala Tyr Ser Tyr Pro Gln Asp Asp Pro Thr
        195                 200                 205
Ser Thr Phe Thr Cys Pro Ser Gly Ser Thr Asn Tyr Arg Val Val Phe
    210                 215                 220
Cys Pro Asn Gly Val Thr Ser Pro Asn Phe Pro Leu Glu Met Pro Ala
225                 230                 235                 240
Ser Asp Glu Glu Ala Lys
                245
```

<210> 16
<211> 529
<212> PRT
<213> Helianthus annuus

<400> 16

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Met Glu Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Leu Ser Thr Gln
 1               5                  10                  15
Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys Leu
            20                  25                  30
His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr
        35                  40                  45
Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn
    50                  55                  60
Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr
65                  70                  75                  80
Ala Glu His Val Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln
                85                  90                  95
Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly
            100                 105                 110
Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met
        115                 120                 125
Phe Asn Leu Arg Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp
    130                 135                 140
Val Gln Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu
145                 150                 155                 160
Lys Ser Asn Lys His Gly Phe Pro Ala Gly Val Cys Pro Thr Val Gly
                165                 170                 175
Val Gly Gly His Phe Ser Gly Gly Gly Tyr Gly Asn Leu Met Arg Lys
            180                 185                 190
Tyr Gly Leu Ser Val Asp Asn Ile Val Asp Ala Gln Ile Ile Asp Val
        195                 200                 205
Asn Gly Lys Leu Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe Trp
    210                 215                 220
Ala Tyr Thr Gly Gly Gly Gly Val Ser Phe Gly Val Val Leu Ala Tyr
225                 230                 235                 240
Lys Ile Lys Leu Val Arg Val Pro Glu Val Val Thr Val Phe Thr Ile
                245                 250                 255
Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr Ile Ala Glu Arg Trp Val
            260                 265                 270
Gln Val Ala Asp Lys Leu Asp Arg Asp Leu Phe Leu Arg Met Thr Phe
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275 280 285
Ser Val Ile Asn Asp Thr Asn Gly Gly Lys Thr Val Arg Ala Ile Phe
290 295 300
Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn Leu Val Thr Leu Leu Asn
305 310 315 320
Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu Ser Asp Cys Thr Glu Met
325 330 335
Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr Gly Phe Pro Ser Gly Thr
340 345 350
Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe
355 360 365
Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro Ile Ser Lys Arg Gln Phe
370 375 380
Glu Phe Ile Phe Glu Arg Met Lys Glu Leu Glu Asn Gln Met Leu Ala
385 390 395 400
Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu Ile Ser Glu Phe Ala Lys
405 410 415
Pro Phe Pro His Arg Ser Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val
420 425 430
Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu Asn Arg Tyr Leu Asn Phe
435 440 445
Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro Phe Val Ser Lys Asn Pro
450 455 460
Arg Glu Ala Phe Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn Ser
465 470 475 480
His Gly Arg Asn Ala Tyr Thr Glu Gly Met Val Tyr Gly His Lys Tyr
485 490 495
Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val Ser Val Lys Thr Lys Val
500 505 510
Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser
515 520 525
Ser

<210> 17
<211> 529
<212> PRT
<213> Helianthus annuus

<400> 17
Met Gln Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Leu Ser Thr Gln
1 5 10 15
Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys Leu
20 25 30
His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr
35 40 45
Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn
50 55 60
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<210> 41
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<212> DNA
<213> Helianthus annuus

<400> 41
gctgcagctt gcaaaatggg tatgta 26

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International Bureau

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28 December 2000 (28.12.2000)

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(21) International Application Number: PCT/US00/17090

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(71) Applicants *(for all designated States except US)*: PIONEER HI-BRED INTERNATIONAL, INC. [US/US]; 800 Capital Square, 400 Locust Street, Des Moines, IA 50309 (US). CURAGEN CORPORATION [US/US]; 13th floor, 555 Long Wharf Drive, New Haven, CT 06511 (US).

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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— *with international search report*

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16 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/78983 A3

(54) Title: SUNFLOWER ANTI-PATHOGENIC PROTEINS AND GENES AND THEIR USES

(57) Abstract: Compositions and methods to aid in protecting plants from invading pathogenic organisms are provided. The compositions of the invention comprise anti-pathogenic genes, including 5' regulatory sequences in the promoter, and proteins encoded by the anti-pathogenic genes. The compositions find use in methods for reducing or eliminating damage to plants caused by plant pathogens. Transformed plants, plant cells, tissues, and seed having enhanced disease resistance are also provided.

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 00/17090

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/29 C12N15/53 C07K14/415 C12N9/06
A01H5/00 A01N65/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 460 753 A (MOGEN INT) 11 December 1991 (1991-12-11)	1,3,4,6, 10,12, 13,15, 17-19, 25-31
Y	the whole document	1,2,10, 11, 13-16, 25-31

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 February 2001	02.03.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Maddox, A

2

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JUNG JEAN-LUC ET AL: "Sunflower (Helianthus annuus L.) pathogenesis-related proteins: Induction by aspirin (acetylsalicylic acid) and characterization." PLANT PHYSIOLOGY (ROCKVILLE) vol. 101, no. 3, 1993, pages 873-880, XP002151834 ISSN: 0032-0889	19
Y	the whole document	1,2,10, 11, 13-16, 25-31
X	EP 0 392 225 A (CIBA GEIGY AG) 17 October 1990 (1990-10-17) page 69 -page 68; examples 16,43	1,3,4,6, 10,12, 13,15, 17-19
X	DATABASE EMBL 'Online! ACCESSION NO: Y10992, 10 February 1997 (1997-02-10) LOULAKAKIS K.A.: "V.vinifera mRNA for osmotin-like protein" XP002151839 the whole document -& PLANT GENE REGISTER PGR97-064. KONSTANTINOS A. LOULAKAKIS (1997) NUCLEOTIDE SEQUENCE OF A VITIS VINIFERA L. CDNA (ACCESSION NO. Y10992) ENCODING FOR OSMOTIN-LIKE PROTEIN (PGR97-064). PLANT PHYSIOL. 113 1464 , 1997 XP002151335	10,13,15

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Inte ional Application No
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Form PCT/ISA/210 (continuation sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 00/17090

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-31 all partially

Isolated nucleic acid molecule and protein representing sunflower PR5 as defined by SEQ ID NOS 1,4, and 7, method for increasing pathogen resistance using said sequences,plants and plant cells incorporating said sequences, promoter represented by said sequence, and protein compositions and their use in pathogen control based on said sequences.

2. Claims: 1-31 all partially

Isolated nucleic acid molecule and protein representing sunflower defensin as defined by SEQ ID NOS 3,6, and 9, method for increasing pathogen resistance using said sequences,plants and plant cells incorporating said sequences, promoter represented by said sequence, and protein compositions and their use in pathogen control based on said sequences.

3. Claims: 1-31 all partially

Isolated nucleic acid molecule and protein representing sunflower RBE as defined by SEQ ID NOS 2,5, and 8, method for increasing pathogen resistance using said sequences,plants and plant cells incorporating said sequences, promoter represented by said sequence, and protein compositions and their use in pathogen control based on said sequences.

INTERNATIONAL SEARCH REPORT

information on patent family members

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